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Supplied free of charge: 250 reprints will be sent to the senior author about three weeks after publication of the article.

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The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1980.

COMMENTARY

The Use of Opiate Analgesics in Cancer Patients [1]

Brian J. Lewis [2,*]

A wise person is one who knows the depth of his or her ignorance. By this criterion, many physicians are less than wise in their use of narcotic analgesics, especially in cancer patients. A simple self-examination will prove the point.

1. What are the benefits or the risks of using a PRN schedule of opiates as opposed to a fixed schedule in patients with protracted or recurrent pain?
2. Among the side effects of tolerance, physical dependence, central nervous system depression, respiratory depression, and constipation, which is a *frequent* and troublesome management problem in advanced-cancer patients during moderate or long-term periods of opiate analgesic use?
3. What has been the experience in England and in the hospice movement in general with the use of oral narcotic analgesics for long-term pain control in patients with advanced cancer?
4. What are the times to onset of action, the times to peak effect, the duration of action, and the half-lives of the opiate analgesics you commonly prescribe?

First, a fixed schedule of an analgesic with the dose titrated to a level which will just produce an adequate level of pain control is likely to be better than the more traditionally used PRN schedule. A PRN schedule requires the patient to endure pain and to complain before receiving medication. In the process, it may set up an operant conditioning where "pain behavior" is rewarded with drug and patients will be more likely to develop a craving and a sense of dependency (1,2). On the other hand, administration of an analgesic at regular intervals sufficient to keep the patient pain-free associates drug use with "non-pain" or well behavior. In an intuitive sense, it would also seem more humane to avoid the "roller coaster" effect of periods of pain relief followed by anxiety about the drug wearing off, the pain returning, and the wait to get a nurse's attention and another dose of medication.

With regard to the second question, a frequent and troublesome side effect of prolonged opiate use in cancer patients is constipation. Accordingly, one should educate each patient receiving narcotic analgesics to be alert to decreases in bowel frequency and to use laxatives as regularly as needed to maintain adequate colon function.

As for the third question, the experience in England and in the hospice movement with oral narcotic analgesics has been quite good. Doses have been titrated to a level of analgesia with an elixir of morphine or other agents. Depression of consciousness, tolerance, and psychologic or physical dependence have not been significant management problems, and patients have required parenteral analgesics only during their few final days, if at all (2). It should also be noted that it requires no special clearance in the United States to prescribe formulations of elixirs for use in these patients.

Finally, with reference to the fourth question, chances are that many do not know the pharmacology of the opiates they prescribe in sufficient detail to have confidence that their prescription habits match the needs of individual patients. Instead, we may cover ourselves by using standard doses or routine schedules that are considered safe or minimum for the general population. We tend to underestimate the pain medicine requirements of the patients and overestimate the dangers of the drugs (3). However, even a "safe" approach may not be sufficient to prevent complications of opiate analgesic use.

Ettinger et al (6), in an article in this issue, describe how a lack of appreciation of the pharmacology of methadone, even when given in a "standard" fashion, resulted in depression of consciousness and respiratory drive in three advanced-cancer patients. Methadone is often used in the treatment of chronic cancer pain. It is effective orally and can be

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TABLE 1.—Commonly prescribed narcotic analgesics: representative pharmacokinetic data*

Drug (trade name)	Route of administration	Mins to onset of peak effect (ref 4)	Duration of action (hrs) (ref 5)	Serum half-life (hrs) (ref 6)
Morphine	im	30–60	4–5	2.6
Meperidine (Demerol)	im	30–50	2–4	3.2
Methadone (Dolophine)	Oral	30–60	3–5	22.4
Oxycodone (Percodan)	Oral	10–15	4–5	—
Hydromorphone (Dilaudid)	Oral or im	15–30	4–5	—
Oxymorphone (Numorphan)	Rectal	15–30	3–6	—

*An extensive literature search and direct query of the manufacturers could not produce data on the half-lives of oxycodone, hydromorphone, or oxymorphone.

given at relatively longer intervals than other agents. However, it would appear from the current report that its prolonged half-life leads to difficulties when frequent changes in administration, especially *increases* in dose or frequency, are required. Other compounds with shorter half-lives would appear to be more suitable (table 1).

It will be unfortunate if Ettinger's paper simply further reinforces the conservative treatment of pain in advanced-cancer patients. Instead, it should be a reminder of the need to school ourselves about supportive therapies to the same degree that we concern ourselves with the details of the oncolytic agents employed for cure or control of cancer. Considering the gaps in available knowledge about the pharmacokinetics of several of the commonly used opiate analgesics (*see* serum half-lives in table 1), it would also appear that additional clinical studies are needed to further refine our use of these drugs.

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Pharmacokinetics and Metabolism of Ftorafur in Man [1,2]

Jessie L. Au, Anne T. Wu, Michael A. Friedman, and Wolfgang Sadée [3,4,*]

SUMMARY

The 5-fluorouracil (5-FU) prodrug, ftorafur (FT), was administered iv to cancer patients at a dose of 2 g/m²/day. Plasma concentrations of FT, 5-FU, and four additional FT metabolites were measured by a high-performance liquid chromatography assay with a sensitivity range between 20 ng of 5-FU/ml and 100 ng of FT/ml. The disposition of FT in man followed two-compartment kinetics. The β-elimination half-life of FT ranged from 6 to 16 hours with a plasma clearance of 22–95 ml/kg-hour. Plasma concentrations of 5-FU generated in vivo were < 0.2% of FT concentrations indicating that 5-FU, if formed intracellularly, may be further metabolized without extensive redistribution. Four additional FT metabolites with the furan portion attached to the 5-FU nucleus were isolated from urine. Physicochemical analysis of these metabolites suggests their structures to be 4′-OH-FT (MH-1), 3′-OH-FT (MH-2), dehydro-FT (MH-4) with a double bond in the furan moiety as suggested by mass spectral data, and a metabolite with altered tetrahydrofuran moiety (MH-3). About 5% of the isolated urinary MH-1 was cleaved to 5-FU by a horse liver thymidine phosphorylase suggesting that a small fraction of MH-1 may be present in the β, D configuration. Since MH-1 plasma concentrations are rather low (≤ 100 ng/ml), it can be assumed that a potential activation of FT to 5-FU via MH-1 represents only a minor pathway in man. MH -4, which persists at higher plasma concentrations than the other metabolites, is chemically more labile than FT. Therefore, MH-4 may participate as a metabolic intermediate in the activation of FT by converting nonenzymatically to 5-FU as proposed for 2′,3′-dehydro-5-fluorodeoxyuridine.

[Cancer Treat Rep 63:343–350, 1979]

Ftorafur (FT) has shown significant activity in several adenocarcinomas with less myelotoxicity than 5-fluorouracil (5-FU) (1). It is considered to be a slow-release form of 5-FU which undergoes metabolic activation in vivo (2–5). Microsomal drug-metabolizing enzymes such as cytochrome P-450 may participate in the mechanism of FT activation, and the liver has been proposed as the primary site of activation (2,5–8). We have recently identified two hydroxylated metabolites of FT, ie, 4′-OH-FT (MH-1) and 3′-OH-FT (MH-2), in rats and rabbits (9). Thymidine phosphorylase was capable of partially converting MH-1 to 5-FU which suggested the presence of the β,D-anomer in the isolated urinary MH-1 metabolite fraction (9). This pathway involving a metabolic intermediate provides one possible mechanism of the in vivo activation of FT to 5-FU. In the present report the pharmacokinetics and metabolism of FT were investigated in cancer patients. In addition to MH-1 and -2, a metabolite with altered tetrahydrofuranyl moiety (MH-3) and a dehydrogenated metabolite (MH-4) were identified. The poten-

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[3]School of Pharmacy (*J. L. Au, A. T. Wu,* and *W. Sadée*) and Cancer Research Institute (*M. A. Friedman*), University of California, San Francisco.

[4]We are grateful to Dr. D. Santi for his advice and help with the phosphorylase study and J. Williams, RN, for her assistance.

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tial role of these metabolites as intermediates in the formation of 5-FU was studied.

MATERIALS AND METHODS

Chemicals.—All chemicals were of analytic reagent grade. FT was supplied by the Chemical Resources Section, Pharmaceutical Resources Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, Md). 2′,3′-Dehydro-5-fluorodeoxyuridine (5-DHFUdR) was received from Dr. T. Khwaja, University of Southern California (Los Angeles). 2-^{14}C-FT, R,S-1-(tetrahydro-2-furanyl)-5-fluoro-2-^{14}C-uracil (specific activity, 46 microcuries/mg) and 2′,5′-^{14}C-FT, R,S-1-(tetrahydro-2-furanyl)-2′,5′-^{14}C-5-FU (specific activity, 33 microcuries/mg) were obtained through the Chemical Resources Section of the National Cancer Institute from the Isotope Synthesis Laboratory, Stanford Research Institute (Menlo Park, Calif).

Apparatus.—Analytic techniques included high-performance liquid chromatography (HPLC), gas chromatography (GC), GC-mass spectrometry (GC-MS), and proton nuclear magnetic resonance (^{1}H-NMR). Details of the apparatus and methods were described previously (9).

Patient protocol.—Five patients with various carcinomas were studied (table 1). The patients were randomly recruited from an ongoing phase II clinical trial with FT conducted by the Cancer Research Institute, University of California (San Francisco) and the Northern California Oncology Group. FT dissolved in 5% dextrose in water was infused iv over 30 minutes at a dose of 2 g/m^2 as a single chemotherapeutic agent during the first day of therapy and again together with either methyl-CCNU, adriamycin, or mitomycin C on the second day. Urine was collected from patient 5 over 24 hours after the first dose. Serial blood samples were drawn through an indwelling iv catheter into heparinized tubes and centrifuged, and plasma fractions were immediately extracted with ethyl acetate. The organic layer was dried under nitrogen and reconstituted with methanol for subsequent HPLC analysis.

Isolation of ftorafur and its metabolites from urine.—A 24-hour urine sample (1700 ml) was concentrated in vacuo to about 100 ml. The pH of the urine sample was adjusted to 5 with phosphate buffer and the sample was exhaustively extracted with ethyl acetate. MH-1–4 were isolated and obtained in chromatographically pure form using column chromatography and thin-layer chromatography as previously described (9). Metabolite MH-4 was recycled several times through reverse-phase HPLC to achieve complete separation from FT. The quantities obtained were 200, 750, 750, and 50 μg for MH-1–4, respectively. Metabolites were characterized by ultraviolet (UV) absorbance in methanol, ^{1}H-NMR in methanol-d_4 or acetone-d_6 (except for MH-4), GC, and GC-MS following flash methylation with trimethylphenylammonium hydroxide (Pierce Chemical Co, Rockford, Ill). Metabolite fractions (MH-1–4) were hydrolyzed in 6 N HCl at 190°C for 2 hours in sealed ampules to give free 5-FU which was measured by HPLC. This confirms the presence of the unchanged 5-FU moiety in these metabolites.

HPLC assay of ftorafur and its metabolites in plasma.—Plasma samples were extracted with ethyl acetate as previously reported (9). The extraction yield was 85% for FT and MH-4, 60% for 5-FU, 50% for MH-1, 70% for MH-2 and -3, and 30% for 5-fluorouridine (5-FUrd) and 5-fluorodeoxyuridine (5-

TABLE 1.—Patient data

Patient No.	Age (yrs)	Sex	Diagnosis	FT dose (g/day)	2nd chemotherapeutic agent	Levels of— Total bilirubin (mg/100 ml)*	Alkaline phosphatase (IU/liter)†	Serum creatinine (mg/100 ml)‡	BUN (mg/100 ml)§
1	67	M	Primary hepatoma	3.4	Methyl-CCNU	5.9	262	0.9	11
2	66	M	Primary hepatoma	3.0	Adriamycin	10.0	220	1.4	33
3	55	M	Primary hepatoma and pleural effusion	3.9	Mitomycin C	0.5	68	1.0	13
4	64	M	Liver metastasis, pleural effusion, and cirrhosis	3.0	Adriamycin	0.6	61	0.7	7
5	68	F	Liver metastasis	3.0	Methyl-CCNU	0.6	71	0.9	22

*Normal range = 0.2–1.5 mg/100 ml.
†Normal range = 23–71 IU/liter.
‡Normal range = 0.5–1.2 mg/100 ml.
§Normal range = 10–20 mg/100 ml.

FdUrd). The previously published HPLC assay (9) was modified by using 0.01 M pH 4 sodium acetate aqueous buffer:methanol (95:5) at a flow rate of 2 ml/minute and dual UV absorbance detection at 254 and 280 nm. Under these conditions, 5-FU, the sum of MH-2 and -3, FT, and MH-4 as well as 5-FdUrd were measurable. Retention volumes of 5-FU, MH-1–4, FT, and 5-FdUrd were 4.7, 10.6, 14.4, 14.6, 39.0, 32.7, and 10.2 ml, respectively. The characteristic UV absorbance ratios at 254:280 nm of 5-FU, MH-1–4, FT, and 5-FdUrd were 1.45, 0.90, 0.90, 0.80, 0.94, 0.77, and 0.95, respectively. An HPLC record of human plasma extract is shown in figure 1, which demonstrates the relative concentrations of FT and its metabolites. Separation of MH-1 and 5-FUrd from interfering endogenous substrates and of MH-2 from MH-3 was achieved using 0.01 M pH 4 sodium acetate aqueous buffer:acetonitrile (95:5) at a flow rate of 2 ml/minute. Retention volumes of MH-1–3 and 5-FUrd were 7.6, 9.4, 10.0, and 5.2 ml, respectively. However, 5-FU and 5-FdUrd were not separated from interfering endogenous substrates using the latter eluent. Therefore, both eluents were used for complete analysis. Sensitivity of this assay was 20 ng/ml for 5-FU and MH-1–3 and 100 ng/ml for FT, MH-4, 5-FUrd, and 5-FdUrd. Standard curves of FT and 5-FU were linear over a wide range of concentrations. MH-1 was quantitated using standard curves constructed with [2-^{14}C]4′-OH-FT and MH-2 and -3 were quantitated using standard curves constructed with [2-^{14}C]3′-OH-FT. MH-4 was quantitated using standard curves of FT assuming identical chromatographic properties and extinction coefficients at 280 nm.

Enzymatic phosphorolysis of ftorafur and its metabolites.—Horse liver thymidine phosphorylase (0.1 unit/mg, Pipep Co, Paris, France) and rat liver uridine phosphorylase, purified according to Kraut and Yamada (10), were kindly provided by Dr. D. Santi (University of California, San Francisco). Incubation of FT and its metabolites (10–25 μM) in 0.1 M pH 6.0 potassium phosphate buffer was performed with both enzymes separately at 25°C at conditions which afforded complete conversion of 25 μM 5-FdUrd to 5-FU within 2–4 hours. Similar incubations were carried out without enzymes to rule out any nonenzymatic degradation which might have occurred during the incubation period. Disappearance of substrate and 5-FU formation were monitored by HPLC.

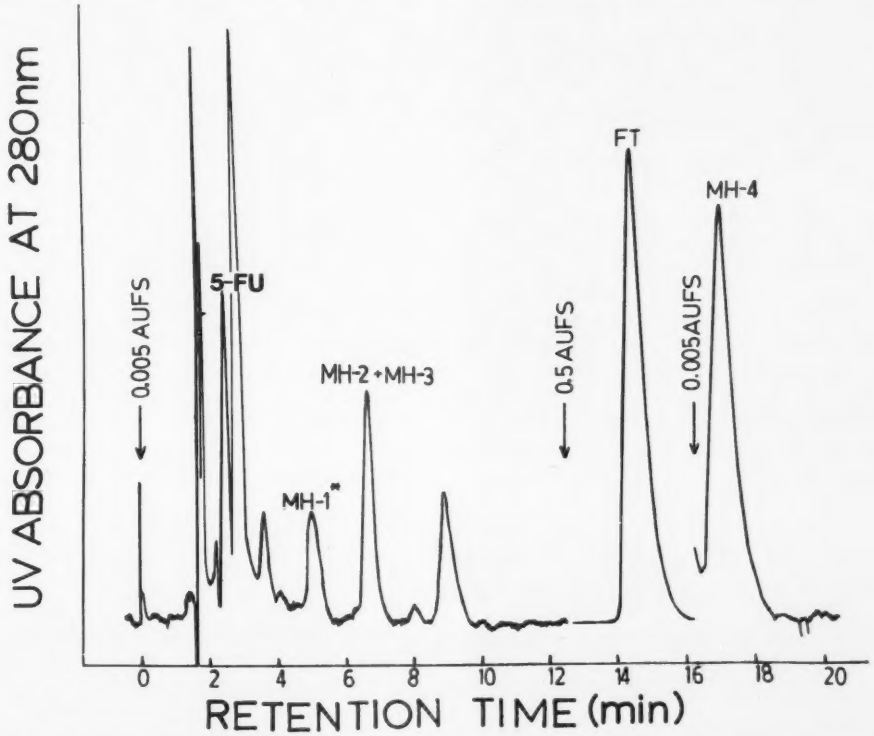

FIGURE 1.—HPLC record of a plasma sample obtained 2 hrs after iv administration of FT at 2 g/m² to a cancer patient (No. 5). *Peak = MH-1 and an interfering endogenous substrate; AUFS = absorbance units, full scale.

Pharmacokinetic analysis.—Pharmacokinetic parameters of FT were obtained by fitting the experimental data with the FITFUN program (Prophet, First Data Computer, Cambridge, Mass) utilizing a two-compartment first-order kinetic model.

RESULTS

Characterization of Metabolites

Metabolites MH-1 and -2.—These metabolites are identical to 4'-OH-FT (MH-1) and 3'-OH-FT (MH-2) previously isolated from rabbit urine (9) as confirmed by chromatography, UV absorbance maxima, ^{1}H-NMR, and GC-MS.

Metabolite MH-3.—This metabolite has been observed in human but not in rat or rabbit plasma and urine. It can be hydrolyzed to 5-FU under strongly acidic conditions and has a UV absorbance maximum at 270 nm which suggests presence of the intact 5-FU nucleus. Mass spectral and ^{1}H-NMR data indicate that the tetrahydrofuranyl moiety is altered in MH-3. The GC-MS spectrum of permethylated MH-3 gave two ion peaks at m/e 244 (M^+, 17%) and m/e 101 (100%), which also occur in the spectra of MH-1 and -2 (9). These ions indicate metabolic insertion of one oxygen atom and addition of two methyl groups by flash methylation. However, the major ion at m/e 69 in the spectra of MH-1 and -2 associated with the tetrahydrofuran moiety (9) is absent in the spectrum of MH-3. The C-6-H signal in the ^{1}H-NMR spectrum of MH-3 is shifted upfield (d, δ7.46 ppm, J_d = 6.4 Hz) when compared to 5-FU (d, δ7.63 ppm, J_d = 6 Hz) while the C-6-protons of 5-FU derivatives with tetrahydrofuran substituent in N-1 are shifted downfield (9). The anomeric proton in C-2' of FT and its hydroxylated metabolites is absent in the spectrum of MH-3; instead, a singlet with the intensity of about two protons appears at δ5.55 ppm. One possible structure consistent with these data is 5-FU-CH_2-CO-CH_2-CH_2OH; however, proof of structure of MH-3 remains to be obtained.

Metabolite MH-4.—This metabolite is more lipophilic than FT based on their relative chromatographic properties. A UV absorbance maximum at 268 nm, as well as acid hydrolysis to give 5-FU, proves the presence of the intact 5-FU moiety. MH-4 was quantitatively converted to 5-FU within 30 minutes under acid conditions, ie, pH 1 and room temperature, whereas only 20% of FT was hydrolyzed to 5-FU under identical conditions. MH-4 also slowly decomposed to 5-FU when stored in methanol or acetone at 4°C. The isolated amount of this metabolite was insufficient for ^{1}H-NMR studies. However, the GC-MS data of permethylated MH-4 give strong supportive evidence for a dehydrogenated metabolite of FT. While no molecular ion was observed, the fragment ions at m/e 144 (100%), 69 (62%), and 68 (60%) characterize both the 5-FU and the dihydrofuran moieties of MH-4 (fig 2). The large abundance of the radical cation at m/e 144, which is small in the spectrum of FT, can arise from formation of stable uncharged furan following proton and charge transfer to the 5-FU ring. The furan radical cation at m/e 68 is generated when the charge remains in the furan moiety. This transition cannot occur with MH-1 and -2 due to lack of the preformed double bond, and the ion at m/e 68 is absent in the spectra of MH-1 and -2. The GC-MS spectrum of permethylated MH-4 was compared to an identically treated sample of 5-DHFUdR which also gave no parent ion, but again fragments at m/e 144 (74%), 112 (38%), and 113 (30%) (equivalent to m/e 68 and 69 of MH-4 plus $-CH_2OCH_3$). An additional peak at m/e 81 (100%) was generated from m/e 113 by loss of CH_3OH. Equivalence of the mass spectra of MH-4 and 5-DHFUdR provides further evidence for the presence of the double bond in the dihydrofuran ring of MH-4. Its exact location, however, cannot be inferred from mass spectral data.

Kinetics of ftorafur and its oxidative metabolites.—The plasma concentration-time curves of FT and its metabolites following the first dose of FT to a representative patient are shown in figure 3. Plasma FT concentrations declined biexponentially, indicating a two-compartment disposition. Pharmacokinetic parameters of FT in these patients are summarized and compared to literature values in

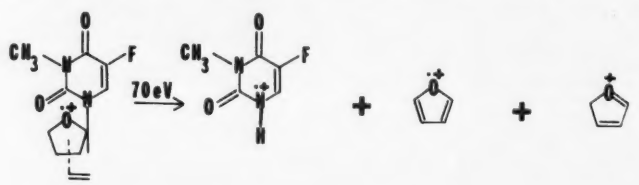

FIGURE 2.—Proposed mass fragmentation of permethylated MH-4.

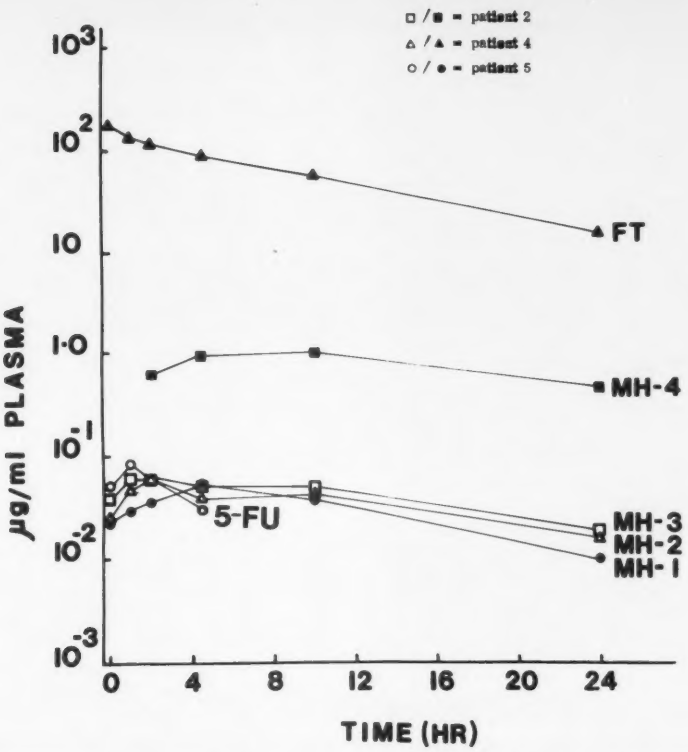

FIGURE 3.—Plasma concentration-time curves of FT and its metabolites following iv administration of FT at 2 g/m² to a representative patient (No. 5). 0 time = end of 30-min infusion. 5-FU concentrations were corrected for nonmetabolically generated 5-FU as described in the text.

table 2. Of the two patients (Nos. 1 and 2) with severe liver damage, only patient 1 displayed a prolonged half-life of 16 hours. Plasma samples from patients 1–4 obtained after the second dose of FT on the following day showed FT concentrations similar to those of the first day of treatment. This rules out significant accumulation of FT in these patients as well as major changes of FT disposition due to repeated dosing or co-administration of other antineoplastic agents, ie, methyl-CCNU, adriamycin, and mitomycin C. The relative ratios of metabolite concentrations at 4 hours after the FT dose were variable from one patient to another (table 3). MH-4 was the major detectable circulating metabolite in all patients, reaching plateau concentrations at approximately 4 hours after the dose which were maintained with little (patient 5, fig 3) or no apparent decline over the 24-hour observation period.

Kinetics of 5-fluorouracil.—Plasma concentrations of 5-FU and FT measured by HPLC in five patients are shown in figure 4. The observed 5-FU concentrations could have arisen from FT metabolism, chemical decomposition of FT during analysis (0.03%) (11), or contamination of the FT dosage form with 5-FU (0.15%). The observed initial rapid decline of 5-FU concentrations partially resulted from the 5-FU contamination of the FT dose. The corrected plasma 5-FU concentrations[5] peaked at 1–2 hours after FT administration. In patient 1, the corrected 5-FU concentrations were below assay limits (≤ 20 ng/ml), while in vivo 5-FU formation was evident in other patients; however, 5-FU concentrations dropped to < 20 ng/ml after 8 hours in all patients. Generally, low 5-FU plasma concentrations are consistent with our previous observations in rats and rabbits (9,11). Neither 5-FUrd nor 5-FdUrd was detectable (≤ 100 ng/ml) in the plasma samples from all five patients.

[5]Corrected 5-FU concentrations (5-FU concentrations resulting from FT metabolism) = [observed values at time t]–[$Cp^{0}_{5\text{-}FU}e^{-k_d t}$]–[0.03% × FT concentrations at time t]; $Cp^{0}_{5\text{-}FU}$ = plasma 5-FU concentrations resulting from a 30-minute infusion at a dose of 0.15% × FT dose (the plasma clearance value used in the calculations was taken from the literature: 2.1 liters/kg-hour [12]); k_d = first-order elimination rate constant, literature value = 4.2 $hour^{-1}$ (12).

TABLE 2.—Pharmacokinetic parameters of FT in cancer patients*

Patient No.	Elimination half-life† (hrs)	Plasma clearance, V_{CL}‡ (ml/kg-hr)	$V_d\beta$§ (liter/kg)
1	16	22	0.51
2	7.5	53	0.58
3	9.4	37	0.49
4	6.0	95	0.78
5	7.6	35	0.39
Range	6–16	22–95	0.4–0.8
Literature values¶	17 (13)	54 (14)	0.4 (14)
	6.7 (14)	50 (15)	0.7 (15)
	10 (15)		
	8.8 (16)		

*Plasma concentration-time curves of FT were computer-fitted to a biexponential function $Cp = Ae^{-\alpha t} + Be^{-\beta t}$ using weighted least-squares method (weight = $1/Cp^2$); β phase = elimination of FT from the body and β = 1st-order elimination rate constant.

†Half-life = $0.693/\beta$.

‡V_{CL} = dose/AUC where AUC is the area under the plasma concentration-time curve from time when infusion was started to time infinity and was calculated by the trapezoidal rule. AUC during the infusion phase = Cp at the end of infusion × infusion time × ½.

§$V_d\beta$ = volume of distribution of FT during the elimination phase = V_{CL}/β.

¶Literature values are obtained either directly from the references or calculated from the data given. Nos. in parentheses = ref Nos.

Nucleoside phosphorylase incubations.—Complete phosphorolysis of 5-FdUrd to 5-FU was observed with both uridine and thymidine phosphorylases. There was no detectable nonenzymatic decomposition of FT and MH-1–4 to 5-FU during the incubation. MH-1 was converted to 5-FU only to the extent of 5% by thymidine phosphorylase within 24 hours. FT and MH-2–4 were not substrates for thymidine phosphorylase. Uridine phosphorylase was inactive against FT and MH-1–4.

DISCUSSION

Ftorafur Conversion to 5-Fluorouracil Measured in Plasma

The observed pharmacokinetic parameters of FT in cancer patients are consistent with those of previous reports (13–15). However, 5-FU plasma concentrations obtained in this study are considerably lower than those observed by Hall et al (13), Hills et al (14), Benvenuto et al (16), and Fujita and Kimura.[6] The sensitive and specific HPLC assay of 5-FU in this report represents a minor modification of our previously published procedure (9). This HPLC method has been independently verified with a column-chromatography GC-MS assay confirming the very low 5-FU plasma concentrations after FT administration in rats and rabbits (9,11). The sensitivity of the present HPLC method of 5-FU detection represents a significant advance over similar previous assays. The high 5-FU plasma concentrations reported by other groups can be caused by several factors. FT is chemically labile and slowly decomposes to 5-FU upon storage and possibly during extractions using high salt concentrations (14). While such decomposition is quite small, it may be significant due to the very large FT:5-FU concentration ratios (1000:1) in the biologic samples. The hydroxylated FT metabolites also rapidly converted to 5-FU in plasma at 37°C (16). In addition, the dehydrogenated metabolite, MH-4, first described here is chemically more labile than FT itself and appreciably decomposes to 5-FU during storage. Acidic conditions employed during analysis by Fujita and Kimura[6] and Freudenthal and Emmerling

[6]Fujita H, and Kimura K. In vivo distribution and metabolism of N_1-(2-tetrahydrofuryl)-5-fluorouracil (FT-207). Presented at the Japanese Congress of Chemotherapy, Gifer City, Japan, 1977.

TABLE 3.—Plasma concentrations of FT and its metabolites in cancer patients 4 hrs after iv dose of 2 g of FT/m²

Patient No.	Plasma concentration of— FT (μg/ml)	Corrected 5-FU* (ng/ml)	MH-1 (ng/ml)	MH-2 (ng/ml)	MH-3 (ng/ml)	MH-4 (ng/ml)
1	85	20	20	24	22	Not measured
2	65	94	61	70	42	259
3	77	20	Not measured	53	45	647
4	43	20	52	63	86	376
5†	91	31	53	38	51	950

*5-FU concentrations were corrected for nonmetabolically generated 5-FU as described in the text.
†Concentrations at 4.5 hrs after FT administration.

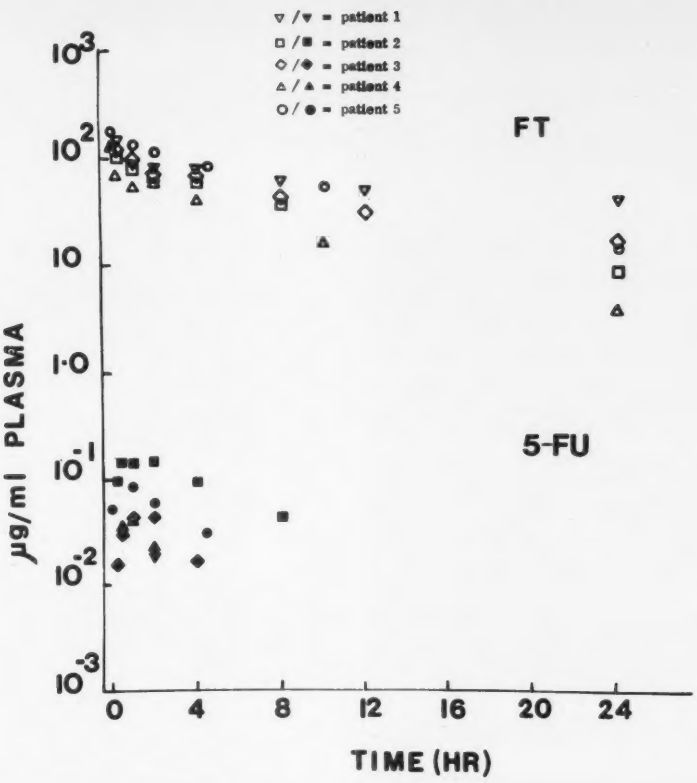

FIGURE 4.—Plasma concentrations of FT and 5-FU in cancer patients following iv administration of FT at 2 g/m². 0 time = end of 30-min infusion. 5-FU concentrations were corrected for nonmetabolically generated 5-FU as described in the text.

(17) rapidly convert MH-4 to 5-FU and, therefore, introduce an error in determining 5-FU concentrations. Hall et al (13) and Benvenuto et al (16) reported plasma 5-FU concentrations of 1.7 µg/ml up to 96 hours after a rapid iv infusion of FT at a dose of 4–5 g/m². The theoretic dose of 5-FU infusion needed to maintain such concentrations in a 70-kg man over a 96-hour period is 24 g of 5-FU or the equivalent of 37 g of FT, assuming complete conversion to 5-FU. Thus, the reported high 5-FU plasma concentrations are only possible if 5-FU metabolism is significantly inhibited by FT administration. No such evidence was presented (16). We propose that the high 5-FU concentrations are, in fact, representing (in part) concentrations of the dehydrogenated FT metabolite (MH-4) described here. The plasma concentrations of MH-4 reached a plateau at 4 hours after the FT dose and remained essentially constant for up to 24 hours. This kinetic behavior of MH-4 is consistent with the 5-FU concentrations reported by Benvenuto et al (16).

The measured 5-FU plasma concentrations following FT doses in our study are significantly below those observed during slow iv infusions of 5-FU at therapeutic doses over several days (30 mg/kg/day, steady state concentration = 100–400 ng/ml) (18,19). If one maintains that FT is a slow-release formulation of 5-FU, then it follows that intracellularly released 5-FU may be further metabolized without reaching the general circulation in any appreciable quantity. Thus, the mechanism of activation of FT to 5-FU might be a determinant of its selective tissue toxicity.

Oxidative Metabolism of Ftorafur

In a previous study (9), the MH-1 metabolite fraction isolated from rabbit urine was hydrolyzed to the extent of 20% to 5-FU by thymidine phosphorylase. The rather low conversion (5%) of MH-1 isolated from human urine may have been caused by a more rapid metabolism of the β,D-enantiomer

prior to excretion in man when compared to rabbits. The low extent of enzymatic 5-FU formation together with the presence of rather low plasma concentrations of MH-1 (≤ 100 ng/ml) suggest that hydroxylation followed by phosphorolysis may represent only a minor activation pathway of FT in man. The pharmacologic significance of MH-2 and -3 remains unknown.

Dehydrogenation of FT to MH-4 results in a chemically more labile metabolite which could undergo nonenzymatic activation to 5-FU in vivo. Kent and Heidelberger (20) have recently studied the mechanism of activation of a dehydrogenated 5-FU analog, ie, 5-DHFUdR, and concluded that nonenzymatic conversion to 5-FU was responsible for its in vitro and in vivo cytotoxicity. Plasma concentrations of MH-4 were the highest among all FT metabolites and were maintained for up to 24 hours after the FT dose. Contribution of this lipophilic metabolite to 5-FU formation and the neurotoxicity associated with FT remain to be determined. Further routes of FT activation cannot be excluded at present.

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Combined Radiotherapy and Multidrug Chemotherapy for Advanced Head and Neck Cancer: Results of a Radiation Therapy Oncology Group Pilot Study [1,2]

Karen K. Fu,* Ivan J. Silverberg, Theodore L. Phillips, and Michael A. Friedman [3,4]

SUMMARY

Between February 1976 and June 1977, 15 patients with advanced inoperable squamous cell carcinoma of the head and neck were entered in a pilot study sponsored by the Radiation Therapy Oncology Group using combined radiotherapy and multidrug chemotherapy. Chemotherapy consisted of cyclophosphamide, vincristine, and bleomycin (during radiotherapy) and cyclophosphamide, methotrexate, and bleomycin (after radiotherapy). At the time of last followup or of death, the disease had been controlled at the primary site in eight of the 15 patients (53%), in the neck in 12 patients (80%), and in all sites in seven patients (47%). Followup time ranged from 2 to 24+ months with a median of 7 months. Acute toxic effects of the combined treatment, primarily enhanced radiation mucositis and infection, were severe, and three patients had fatal complications. Although the combination chemotherapy may have enhanced the tumor response to radiotherapy in some of these patients, treatment morbidity and complications were prohibitive for the treatment program to progress to a randomized trial.

[Cancer Treat Rep 63:351–357, 1979]

Radiotherapy as a single treatment modality can control some advanced head and neck cancer; however, complete response rates are low. In our experience with radiotherapy alone, the initial control rates for the primary lesions in stage III and IV disease were 31% for carcinoma of the supraglottic larynx, 26% for carcinoma of the floor of the mouth, and 12% for carcinoma of the oral tongue (1–3). Chemotherapy alone is seldom used as the primary treatment in these diseases. When tried, the response rates were disappointingly low and durations of response tended to be short (4).

Chemotherapeutic agents in combination with radiotherapy have the potential advantages of increased tumor control rate within the irradiated volume and sterilization of occult microscopic metastases outside the radiation field. However, clinical trials conducted to date using combination radiotherapy and single-agent chemotherapy in advanced head and neck cancer have not yielded definitive findings. When methotrexate was used as an adjuvant to radiotherapy, randomized studies reported by Friedman et al (5), Kramer (6), and Knowlton et al (7) demonstrated no significant improvement in the local control rate or survival. In a randomized study using 5-fluorouracil and radiotherapy reported by Lo et al (8), although the local control rate and survival were better in the combined treatment group than in the group treated with radiotherapy alone, the improvement was statistically significant only in patients with carcinoma of the oral cavity. In a randomized trial reported by Shanta and Kirshnamurthi (9), signifi-

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cant improvement in the local control rate 8 weeks after radiotherapy was observed when bleomycin was administered concurrently with radiotherapy, but the effect on long-term disease-free survival was unclear. With all of these single-agent combined-modality regimens, initial tumor regression has been more rapid than that observed with radiotherapy alone but none has demonstrated increased long-term local control and disease-free survival to date.

Combinations of multiple drugs for various tumor sites have been studied intensively during the last decade. Increased tumor response rate and survival have been demonstrated over a broad spectrum of tumors including lymphoma and carcinoma of the breast, lung, and ovary when multiple-drug combination chemotherapy was used compared to single-drug chemotherapy. Logically, this principle would also apply to squamous cell carcinoma of the head and neck. Preliminary results using combination multidrug chemotherapy and radiotherapy reported by Clifford et al (10) and Vallejo (11) suggest an improved local tumor control rate with combined treatment.

To evaluate toxicity and efficacy of combined radiotherapy and multidrug chemotherapy for advanced head and neck cancer, we undertook a pilot study using cyclophosphamide, vincristine, bleomycin, and methotrexate in combination with conventional radiotherapy under the sponsorship of the Radiation Therapy Oncology Group (RTOG).

MATERIALS AND METHODS

As shown in table 1, the treatment program consisted of two phases in continuum: schedules A and B.

Schedule A.—Radiotherapy was delivered in five daily 180-rad fractions/week with a planned 2-week rest after 1800 rads in 2 weeks. Concurrent chemotherapy consisted of cyclophosphamide, vincristine, and bleomycin administered iv during the first week of radiotherapy. Thereafter, bleomycin alone was administered twice a week until completion of radiotherapy.

Schedule B.—Combination chemotherapy using cyclophosphamide, methotrexate, and bleomycin was administered 6–8 weeks after completion of schedule A.

Radiotherapy was given via a 4-MeV linear accelerator at approximately 200 rads/minute with an 80-cm treatment distance. Treatment fields usually included the primary tumor and its adjacent tissue together with the entire neck. The treatment plan called for delivery of 6500–7500 rads to the primary tumor, adjacent tissue, and involved lymph nodes and 5000 rads to the uninvolved lymph node areas.

Patient entry was restricted to patients with inoperable stage III or IV squamous cell carcinoma of the oral cavity, pharynx, or larynx as defined by the 1977 American Joint Committee for Cancer Staging and End-Results Reporting (12). These patients were considered inoperable because the disease was technically inoperable (incurable by surgery as determined by the referring ear, nose, and throat surgeons) or the patient had medical contraindications to surgery. For a patient to be eligible for the study, the disease had to be measurable, no prior radiotherapy or chemotherapy could have been given, and there could be no second cancer. Patients had to have a minimal Karnofsky performance status of 60% and adequate renal, hepatic, hematopoietic, and pulmonary function.

RESULTS

Between February 1976 and June 1977, 15 patients with advanced inoperable squamous cell carcinoma of the head and neck seen at the University of California, San Francisco Medical Center, were entered into this RTOG pilot study. According to the 1977 American Joint Committee staging system (12), 13 patients had stage IV disease and two patients had stage III disease. A summary of the clinical data of these 15 patients is shown in table 2.

Eight females and seven males ranging in age from 37 to 66 years with a mean of 55 years were treated. The Karnofsky performance status was 90% in two patients, 80% in five patients, 70% in two patients, and 60% in six patients.

All patients completed treatment schedule A. The total dose of external radiotherapy ranged from 5400 to 7460 rads with a mean of 6640 rads. One patient had an interstitial implant with iridium 192 to the oral tongue with 2595 rads delivered following 5540-rad external irradiation. One patient underwent salvage surgery after 5400 rads.

Seven patients began treatment schedule B. Of these seven patients, the disease progressed in three during schedule B, one died of cerebral hemorrhage secondary to thrombocytopenia with persistent disease, one died of sepsis without autopsy evidence of disease, one died in an automobile accident without evidence of disease, and only one patient completed the treatment. Of the other eight patients who did not receive treatment schedule B, one died at the end of treatment schedule A and the others received no further chemotherapy due to severe mucositis and general debilitation or because they refused further treatment.

Table 3 shows the disease activity of the primary

TABLE 1.—Treatment program

Treatment	Week 1 Day— 1	2	3	4	5	6,7	Week 2 Day— 8	9	10	11	12	13,14	Weeks 3–4	Weeks 5–9
Schedule A: remission induction														
Cyclophosphamide (750 mg/m² iv)	×												No treatment	Bleomycin (5 units iv twice a wk) radiotherapy (180 rads/day, 5 days a wk) until completion of radiotherapy
Vincristine (1.4 mg/m² iv)		×												
Bleomycin			15 units*	15 units*	15 units*		5 units†		5 units†					
Radiotherapy (180 rads/day)	×	×	×	×	×		×	×	×	×	×			
Schedule B: remission consolidation														
Cyclophosphamide (500 mg/m² iv on Day 1)														
Methotrexate (25 mg/m² iv on Days 1, 8, 15, and 22)														
Bleomycin‡ (15 units iv weekly beginning on Day 1)														

Repeat q4wks × 6 cycles

* Iv infusion.
† Iv push.
‡ Limit total dose of bleomycin to 400 units.

TABLE 2.—Summary of clinical data of patients treated with a combination radiotherapy and multidrug chemotherapy program

Patient No.	Age/ sex	Disease site	Stage	Maximum mucositis*	Complications	Status at last followup†
1	66/F	Oral tongue	$T_4N_{2a}M_0$	4+	Cerebral hemorrhage secondary to thrombocytopenia	Dead with disease at 5+ mos
2	63/F	Nasopharynx	$T_2N_{2b}M_1$	2+	Necrosis of mandible	Dead with metastases only at 12 mos
3	51/F	Floor of mouth	$T_4N_0M_0$	3+	—	Dead with disease at 5+ mos
4	53/F	Oropharynx	$T_4N_{2b}M_0$	3+	—	Dead without disease at 6+ mos (automobile accident)
5	51/M	Hypopharynx	$T_4N_{3b}M_0$	2+	—	Dead with disease at 4+ mos
6	36/M	Floor of mouth	$T_4N_{2a}M_0$	4+	Bacterial pneumonia and fatal sepsis	Dead with disease at 2 mos
7	54/F	Base of tongue	$T_4N_0M_1$	2+	Fatal sepsis	Dead without disease at 5+ mos
8	61/M	Posterior pharyngeal wall	$T_3N_1M_0$	4+	Bacterial pneumonia	Dead without disease at 5+ mos
9	37/M	Paranasal sinuses	$T_4N_{3b}M_0$	2+	—	Alive with equivocal disease at 24+ mos
10	64/M	Hypopharynx	$T_2N_{2a}M_0$	2+	—	Alive without disease at 24+ mos
11	62/F	Base of tongue	$T_4N_0M_0$	2+	—	Dead with disease at 8+ mos
12	50/F	Supraglottic	$T_4N_{3b}M_0$	3+	Fistula after salvage surgery	Alive with stomal recurrence after salvage surgery at 22+ mos
13	64/F	Oral tongue	$T_4N_0M_0$	4+	Bacterial pneumonia	Dead with disease at 16+ mos
14	59/M	Base of tongue	$T_3N_1M_0$	3+	Bacterial pneumonia; necrosis of mandible	Alive without disease at 18+ mos
15	54/M	Base of tongue	$T_2N_{3b}M_0$	2+	—	Dead without disease at 7+ mos

**See* Results section for definitions.
†As of Dec 1978.

TABLE 3.—Disease activity of the primary lesion and cervical lymph nodes according to time after radiotherapy

Disease site	Disease activity	No. of patients at— End of radiotherapy	3 mos	Last followup
Primary lesion	NED*	6	8	7
	Equivocal	4	2	1
	> 50% regression	4	2	1
	< 50% regression	1	0	1
	Progression	0	2	4
	Stomal recurrence after salvage surgery	0	0	1
Nodes	NED*	9	11	12
	Equivocal	1	1	0
	> 50% regression	3	1	1
	< 50% regression	2	0	1
	Progression	0	1	1

*NED = no evidence of disease.

lesion and cervical lymph nodes according to the time after radiotherapy. At completion of treatment schedule A, 14 of the 15 patients showed > 50% regression of the primary lesion, including six patients who showed no evidence of disease and four patients who had only equivocal evidence of disease. One patient who had a < 50% regression of disease in the primary site died shortly after the end of radiotherapy. At 3 months after radiotherapy, eight patients were free of disease at the primary site, two patients had equivocal evidence of disease, two patients had persistent disease, and two patients had progressive disease. At the time of last followup, seven patients remained free of disease in the primary site. One patient with extensive squamous cell carcinoma involving all the paranasal sinuses was free of disease clinically while roentgenograms showed persistent bony destruction although there was no evidence of progression. Thus, control of the primary lesion was achieved in eight of 15 (53%) of the patients at the time of last followup.

Eleven patients presented initially with cervical lymph node metastasis. At completion of treatment schedule A, five of these patients had complete regression of cervical adenopathy and one patient had equivocal persistent adenopathy. Three patients had > 50% regression of disease; one of these underwent radical neck dissection as salvage therapy and was free of disease in the neck at the time of death (due to pneumonia) 7 months after treatment. Two patients had < 50% regression of cervical lymphadenopathy; one of these subsequently underwent salvage surgery and she remained free of disease in the neck at 22+ months. At the time of last followup, disease in the neck appeared to be controlled in 12 of 15 (80%) of the patients including the four patients who had had no cervical lymph node disease on presentation.

Two patients presented with lung metastasis. One was a patient with biopsy-proven lung metastasis who received radiotherapy (7020 rads in 40 fractions in 74 days) to a solitary lung nodule during treatment of the primary lesion. When she died of sepsis 5 months later, there was no autopsy evidence of residual disease in the lung. The other patient, who had a nasopharyngeal primary, remained free of disease in the primary site and in the neck but died with progressive lung disease 5 months after therapy. One patient with carcinoma of the base of the tongue developed a biopsy-proven metastasis to the anterior mandible 4 months after completion of schedule A. He was then re-treated with radiotherapy (700 rads in one fraction per week × 6 weeks) in conjunction with misonidazole (2 g/m^2 × 6) and vitamin C (6–15 g/day) which he took voluntarily during his treatment. His metastasis in the mandible regressed completely at the end of the combined regimen of irradiation, misonidazole, and vitamin C. He remained free of disease 18 months after his initial treatment and 14 months after completion of therapy for his metastatic disease.

Although we had intended to investigate the role of further combination chemotherapy after completion of radiotherapy in the consolidation of complete responses and inducement of further tumor regression in patients who had achieved a partial response, only seven patients started treatment schedule B and only one of those seven completed the prescribed treatment. None of the patients who had only partial response at the end of radiotherapy achieved further tumor regression with subsequent consolidation combination chemotherapy.

At the time of this analysis (December 1978), three patients were alive without disease at 24, 24, and 18 months; one patient was alive with stomal recurrence after salvage surgery at 22 months. Eleven patients had died, four without disease at 7, 6, 5, and 5 months and seven with disease at 16, 12, 8, 5, 5, 4, and 2 months. Thus, six of the 15 patients (40%) survived ≥ 1 year.

At the time of last followup or of death, seven of the 15 patients (47%) were free of disease at all sites. The duration of complete and partial responses ranged from 3 to 24+ months with a mean of 8+ months.

Treatment complications and toxic effects were formidable. Three patients had fatal complications; one died of cardiovascular accident secondary to severe bone marrow suppression during treatment schedule B. Two patients died of pneumonia and generalized sepsis: one at the end of treatment schedule A and one during treatment schedule B. Thus, the mortality rate was 20% (three of 15 patients).

The acute toxic effects were enhanced radiation mucositis and infection. During treatment schedule A, the maximum degree of mucositis was 2+ (patchy) in seven patients, 3+ (confluent) in four, and 4+ (severe mucositis requiring hospitalization) in four. In spite of a planned 2-week break after the first 1800 rads, ten patients required additional breaks of 4–15 days and one patient developed severe mucositis and aspiration pneumonia requiring hospitalization and cessation of treatment for 49 days. The majority of the patients (nine of 15) developed the maximum degree of mucositis when the irradiation dose had reached 3500 and 4500 rads. Although the degree of mucositis was more severe than usually observed with radiation alone, the degree of skin reaction did not appear to be increased by the concurrent administration of drugs.

Only two patients developed patchy moist desquamation: one at 4140 rads and one at 6120 rads. The other patients had erythema and dry desquamation only.

In addition to the two patients who died of sepsis, three patients developed bacterial pneumonia and eight developed oral and pharyngeal moniliasis during treatment schedule A.

All of the patients tolerated the chemotherapy as specified in the protocol during treatment schedule A. Although two patients developed skin rash, probably secondary to bleomycin, no pulmonary toxicity due to bleomycin was detected. Most of the seven patients who received further chemotherapy on completion of schedule A required reduced drug doses due to low blood cell counts. One patient died of cardiovascular accident secondary to severe bone marrow suppression.

Late normal tissue complications developed in three patients. One patient with carcinoma of the nasopharynx received 7029 rads in 39 fractions over 70 days and developed necrosis of the mandible at 4 months. One patient developed bone necrosis following re-treatment with radiotherapy (700 rads in one fraction per week × 6 weeks) in conjunction with misonidazole for metastasis to the anterior mandible. One patient developed esophageal-cutaneous fistula after salvage surgery. She had received 5400 rads to the primary lesion and involved neck nodes.

DISCUSSION

This pilot study was carried out to determine the feasibility of multiple-drug administration during and after radiotherapy for advanced head and neck cancer. From the results presented above, it is evident that the acute toxic effects of the combined treatment were severe. The addition of cyclophosphamide, vincristine, and bleomycin during radiotherapy markedly enhanced the acute radiation effects most prominently in the mucous membranes. In spite of a planned 2-week break after a dose of 1800 rads, the majority of patients required additional breaks. Four patients developed severe confluent mucositis necessitating hospitalization. The degree of enhanced mucositis appeared to be greater than that observed when bleomycin alone was administered concurrently with radiotherapy (13). Although cyclophosphamide and vincristine by themselves do not appear to enhance radiation mucositis, our results suggest that when they are combined with bleomycin during the first week of radiotherapy the acute toxicity to the mucous membranes is greatly enhanced. It also appears that the patients were generally more debilitated or immunosuppressed during the concurrent multidrug and radiotherapy administration. The average weight loss during treatment schedule A was 10.4 lbs. Four patients developed bacterial pneumonia during radiotherapy and three patients died of complications of treatment.

Late effects of the combined treatment program cannot be adequately evaluated at this time since the longest followup is only 24 months and only four patients are still alive. Although complications such as bone necrosis and esophageal-cutaneous fistula after salvage surgery have been observed, these could have occurred with radiotherapy alone.

In addition to evaluating the normal tissue effects of the combined treatment, we also intended to determine whether the addition of multiple drugs would enhance the tumor response to radiotherapy. Although eight of 15 patients (53%) achieved local control and seven of 15 patients (47%) were free of disease at all sites at the time of last followup or of death, the small number of patients and the limited followup do not permit meaningful evaluation of tumor responses at this time.

During the period of our study, another RTOG pilot study using a combination of vincristine, bleomycin, methotrexate, and cyclophosphamide during radiotherapy was simultaneously carried out by Glick et al (14). Similar to our experience, severe acute toxic effects encountered during treatment led to the discontinuation of the study after 12 patients had been accrued. In contrast to our experience and that of Glick et al (14), Clifford et al (10) found that although the combination of vincristine, bleomycin, cyclophosphamide, and methotrexate administered before and during radiotherapy enhanced radiation mucositis, it was apparently tolerated and the short-term tumor responses were encouraging enough to warrant further clinical trial.

In most clinical studies involving a combination of drugs and radiation in the treatment of head and neck cancer, the drugs were most frequently administered concurrently with the radiotherapy and varying degrees of enhanced radiation mucositis have been observed (15). In a recent study reported by Vallejo (11), when *cis*-dichlorodiammineplatinum(II) and bleomycin were administered before but not during radiotherapy, no enhancement of acute radiation mucositis or skin reaction was seen and the preliminary results on tumor responses were encouraging. Thus, in terms of acute toxicity, it would appear that when drugs are combined with radiation, sequential administration may be preferable to concurrent administration unless a greater tumor response can be demonstrated only with the latter.

In conclusion, although the combination chemo-

therapy as outlined in this treatment program may have enhanced the tumor response to radiotherapy in some of our patients, morbidity and complications of treatment were prohibitive for this treatment program to be used in a randomized trial. The use of more effective, less toxic combinations of existing or new more effective chemotherapeutic agents and radiation using different doses and different time sequences of drug and radiation administration and/or hypoxic cell radiosensitizers needs further clinical trial in the management of advanced head and neck cancer.

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Patterns of Relapse in Patients With Small Cell Carcinoma of the Lung Treated With Adriamycin-Cyclophosphamide Chemotherapy and Radiation Therapy [1,2]

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SUMMARY

Twenty-seven patients with small cell carcinoma of the lung were treated sequentially with induction chemotherapy (adriamycin and cyclophosphamide), radiation therapy (chest and whole-brain), and then maintenance chemotherapy for 2 years. Twenty responding patients were followed to relapse and patterns of recurrence were observed. This combined treatment resulted in a complete remission rate of 80% and a median survival of 565 days in limited-disease patients. Relapse overwhelmingly occurred in the chest, but patients receiving higher-dose radiation (4000–4500 rads in split-course) had a significant prolongation of time to recurrence compared to patients receiving 3000 rads in a single course of radiation (540 versus 270 days). Despite a long mean survival, only one limited-disease patient relapsed outside of the brain or chest, suggesting that chemotherapy had a good protective effect against micrometastatic disease. Three patients relapsed in the brain at 330, 450, and 520 days, suggesting that in future studies the prophylactic whole-brain radiation (3000 rads) should be intensified.

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Radiation and a host of chemotherapeutic treatment programs are successful in palliating patients with small cell carcinoma, but cures remain elusive (1). Adriamycin and cyclophosphamide combined rank high in ability to induce remission and prolong survival in this disease (1–3). Although prophylactic whole-brain radiation is useful in preventing brain relapse (1,2,4,5), the role of chest radiation as an adjunct to chemotherapy remains controversial (4,6,7). In this study, we report our observations on patterns of recurrence in 20 patients treated with adriamycin-cyclophosphamide chemotherapy, whole-brain radiation, and two dose schedules of radiation to the primary site and mediastinum. Hopefully, analysis of treatment-failure patterns will help formulate better treatment strategies.

PATIENT EVALUATION AND RESPONSE CRITERIA

Previously untreated patients with biopsy-proven small cell bronchogenic carcinoma at the Tucson Veterans' Administration Medical Center and the University of Arizona Health Sciences Center were accepted in this pilot study. An assessment was made as to whether the disease was limited (confined to hemithorax to include hilar, mediastinal, and ipsilateral supraclavicular nodes, and pleural disease if included in the radiation field) or extensive (tumor at any other site). Workup included history and physical examination, appropriate chest roentgenograms and tomograms, bone scan, bone marrow examination, brain scan, cbc, and SMA-12 profile. Hepatomegaly or abnormal liver function tests necessitated a liver scan. The above proce-

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dures were repeated at 3–6-month intervals and as dictated by clinical suspicion of recurrence. Complete response (CR) was defined as the disappearance of all clinical evidence of tumor. This did not always mean normalization of the chest roentgenogram since in some cases what appeared to be "radiation fibrosis" in the opinion of the radiologist persisted throughout the course. These were not called clinical CRs, however, unless stability was proven for 6 months. Partial response (PR) was defined as a $\geq 50\%$ reduction in the greatest diameter of a measurable lesion, persisting for 1 month. Survival was measured from entry in the study to death and recurrence time was measured from entry in the study to documented recurrence. At relapse, patients were re-staged in order to determine all sites of relapse. The determination of relapse within the chest in patients with residual fibrosis was made at repeat bronchoscopy or when the suspicious area showed an unequivocal increase in size. In all of these patients relapse was confirmed by the clinical course and/or autopsy results.

THERAPY

Adriamycin (40 mg/m^2) was given as a rapid iv infusion on Day 1; cyclophosphamide (200 mg/m^2) was given orally on Days 3–6. This combination was repeated every 21–28 days as blood cell counts allowed. If the wbc count was $< 3000/mm^3$ or if the platelet count was $< 100{,}000/mm^3$ at 28 days, the subsequent doses were reduced by 25%. In patients receiving split-course radiation, a course of chemotherapy at a 75% dose was administered during the radiation rest period. When the adriamycin dose reached 500 mg/m^2, chemotherapy was continued with cyclophosphamide given at a dose of 200 mg/m^2 on Days 1–5, vincristine given at a dose of 1.0 mg iv on Day 1, and methotrexate given at a dose of 20 mg/m^2 iv on Day 1; q21–28days until 24 months after the start of therapy, at which time treatment was discontinued.

Patients received radiation to the lung primary, mediastinum, and bilateral supraclavicular nodes. Either 3000 rads in ten fractions at 6–8 weeks or 4000–4500 rads in split-course at 6 and 11 weeks (2000–2500 rads/five fractions/week) were administered. In addition, 3000 rads of whole-brain radiation were given in ten fractions during the sixth and seventh week. Patients were randomly assigned to either radiation dose schedule.

RESULTS

Twenty-seven patients were entered in this protocol. The 20 responding patients were followed to recurrence and/or death. One patient survives without evidence of disease at 1140 days and three patients are alive with disease. Sixteen patients are dead, two of causes other than tumor. Of the 14 patients who died from their tumors, eight received an autopsy and in the remaining six a recurrence was established by appropriate clinical studies and biopsy.

Response rates and survival are summarized in table 1. Eight of ten (80%) of the limited-disease patients had a clinical CR and two (20%) had a PR. Three of 17 (18%) of the extensive-disease patients had a CR and seven (41%) had a PR. The median survival of limited-disease patients was 565 days (range, 270–1140) and the median survival of extensive-disease patients was 77 days (range, 37–540) ($P < 0.01$). Survival was related to both stage of disease at the start of treatment and chest radiation dose schedule (table 2). Both patients with limited disease and those with extensive disease survived longer if they received high-dose radiotherapy (470 versus 720 and 252 versus 100 days median survival respectively).

Relapse occurred within the chest radiation field in 70% of the patients and was the only site of relapse in 60% of the limited-disease patients (table 3). Extensive-disease patients also relapsed heavily in the chest, but in only 20% was this the only site of relapse. The three brain recurrences were diagnosed at 330, 450, and 520 days. All three of these patients had normal brain scans at the start of treatment. Only one patient relapsed in the brain

TABLE 1.—Response and survival

Stage	No. of— Patients	CRs*	PRs	Disease progressions	Median survival in days (range)
Limited-disease	10	8	2	0	565 (270–1140)
Extensive-disease	17	3	7	7	77 (37–540)

*CR is discussed in the Patient Evaluation and Response Criteria section.

as the solitary site. Two of the brain relapses were in limited-disease patients. Other sites of relapse occurred in most extensive-disease patients but only in one limited-disease patient despite prolonged periods of survival in the latter group. The other sites were the usual for small cell carcinoma and included liver (four patients), bone (five patients), adrenal glands (one patient), marrow (one patient), pleura (two patients), and spleen (one patient). No testicular or spinal cord metastasis occurred in this study.

The median time to chest relapse in higher-dose split-course radiation patients (540 days) significantly exceeded the median time to relapse in patients receiving 3000 rads in a single course (270 days) ($P < 0.06$, Wilcoxon sign-rank test).

DISCUSSION

The results of this study support other observations (2,3) that similar combined adriamycin-cyclophosphamide-radiotherapy regimens are effective therapy for small cell carcinoma of the lung. Limited-disease patients achieved an 80% response rate in this study with a median survival of 565 days. It is disappointing that all but one of the 11 patients with CR had a late relapse at the site of initial large tumor burden. However, this is a relapse pattern that has been previously described in small cell carcinomas, as well as in other responsive tumors (2,8–10).

Radiotherapy has been employed in the past as an adjunct to chemotherapy with the hope of minimizing the recurrence in sites of large initial tumor burden. The studies are summarized in a recent review by Bunn et al (1). Properly controlled studies are not available to accurately assess the effectiveness of radiation in this regard. Important variables include radiation dose and fractionation and timing in relation to chemotherapy (4,9–11). Obviously, drugs that enhance radiation effectiveness without excessively increasing toxicity are preferred.

Radiation as an adjunct to chemotherapy seemed beneficial in this study. Patients receiving higher-dose split-course radiation survived longer (both limited and extensive disease), and time to chest recurrence was significantly better in the higher-dose radiation group, although the ultimate frequency of chest recurrence was not altered. It should be noted that the radiation given in this study, even in the higher-dose (4500 rads) patients, was short of the potential maximum dose known to be necessary to significantly influence other bronchogenic carcinomas (5000–7000 rads) (12).

Prophylactic whole-brain radiation was of apparent benefit in this study since only three of 20 responding patients relapsed in the brain, considerably fewer than might be expected considering the natural history of the tumor (1). In other studies, 3000 rads of prophylactic whole-brain radiation have at least deferred brain relapse. However, the appearance of brain metastasis in this study in the long-term survivors at a median time to relapse of 300 days suggests the possibility of incomplete sterilization of micrometastasis at the time of initial

TABLE 2.—Median days of survival (range) in responding patients* according to disease stage and chest radiation received

Stage	Treatment (rads)	
	3000	4000–4500
Limited-disease	470 (270–970)	720 (500–1140†)
Extensive-disease	100 (37–450)	252 (60–540)

*5 limited-disease patients and 6 extensive-disease patients received 3000 rads; 5 limited-disease patients and 4 extensive-disease patients received 4000–4500 rads.

†Patient still living without evidence of disease.

TABLE 3.—Patterns of relapse in responding patients

Site	No. of patients (%)	Median days to relapse	Only site of relapse
Chest (rads)	14 (70%)	410	40%
3000	7 (35%)	270	60% of limited disease
4000–4500	7 (35%)	540*	20% of extensive disease
Brain	3 (15%)	330	5%
Other sites†	7 (35%)	450	0

*Difference is significant at $P < 0.06$ level by Wilcoxon sign-rank test.

†Bone, 5 patients; liver, 4 patients; pleura, 2 patients; marrow, 1 patient; spleen, 1 patient; adrenal glands, 1 patient.

treatment. Since two of the three brain-relapsing patients also had concomitant relapse in other sites of disease, it is possible that metastasis occurred after radiation.

Relapses outside of the chest or brain occurred primarily in extensive-disease patients who had large tumor cell burdens at the start of therapy. Despite prolonged survival, only one limited-disease patient relapsed with systemic disease, suggesting that the drug regimen is effective against micrometastasis. Recurrence of disease occurred in six patients after maintenance therapy was stopped at 2 years. In sites of original large tumor burden, chemotherapy was apparently unable to completely sterilize the disease.

Future treatment regimens must address the problems of treatment failure such as those identified in this study. Future protocol design should include further improvement in chemotherapy regimens. New techniques such as the in vitro drug sensitivity stem cell culture recently described by Salmon et al (13) might afford a specificity of drug combination selection for these tumors not possible before. Particularly in limited-disease patients, intensification of radiotherapy using dose schedules in line with those needed to treat other bronchogenic carcinomas should be considered. Alternatively, radiation effectiveness might be improved by delaying radiation therapy until maximum debulking of tumor by chemotherapy. In patients with extensive disease, however, important increases in survival will occur only with more effective chemotherapy. Patients who are expected to survive a relatively long time should receive, in addition, intensified prophylactic whole-brain radiotherapy. By re-evaluation of treatment design in view of patterns of failure, the prospects for curative treatment of this tumor should continue to improve.

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Comparative Randomized Study of Protected Environment Plus Oral Antibiotics Versus Oral Antibiotics Alone in Neutropenic Patients [1]

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SUMMARY

A series of 24 patients with severe neutropenia, most of whom had acute myeloblastic leukemia, were treated in an isolation unit with oral nonabsorbable antibiotics and were compared to 21 similar patients receiving oral antibiotics alone. The frequency of bacterial infections was lower in the patients receiving both isolation and oral antibiotics compared to the patients who received only oral antibiotics. The responses to chemotherapy in terms of remission rates were identical for the two groups.

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The treatment of acute leukemia has many therapeutic approaches and the progress which has been made recently with this disease is not only due to chemotherapy but also to improved supportive care. The optimal dose and duration of chemotherapy are often compromised by severe infections resulting primarily from granulocytopenia and, to a lesser degree, from the impairment of immune mechanisms. These infections are caused by various micro-organisms which arise from the hospital environment and from the patient's endogenous flora.

Control of infection in patients with acute leukemia has included attempts to decrease exposure to these exogenous and endogenous potential pathogens through isolation of the patients and elimination of their intestinal flora (1–6). Several studies have demonstrated that patients can tolerate confinement in isolation units during chemotherapy and that the combination of isolation and sterilization of the gastrointestinal tract is effective in reducing the rate of severe infections in granulocytopenic patients (5–7). The respective value of isolation and gastrointestinal decontamination is less clear; therefore, we have compared the efficacy of orally administered nonabsorbable antibiotics with the efficacy of isolation combined with the same oral antibiotic regimen in reducing the frequency of infection in granulocytopenic patients.

MATERIALS AND METHODS

Patient Selection and Laminar Air Flow Room (LAFR)

Patients were eligible for this study during induction treatment of acute leukemia or during bone marrow aplasia regardless of its origin. All were treated at the Institut Jules Bordet between May 1973 and April 1977. Patients were randomly distributed between two groups: *group 1*—isolation and decontamination, in which isolation in an LAFR and oral nonabsorbable antibiotics were employed; and *group 2*—decontamination alone, in which routine hospital ward care was used in combination with nonabsorbable antibiotics. In group 1, bowel decontamination began 3 days before isolation in the LAFR.

The randomization date corresponded with the beginning of the decontamination and the selected patients entered the LAFR after decontamination was completed. The chemotherapy was only begun after the entry into the LAFR. The patients could leave the LAFR when the clinical situation was favorable, ie, if the bone marrow showed hemato-

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logic remission or myeloid proliferation and maturation, despite the fact that sometimes the wbc count was < 1000/mm^3. Thus, the study began on the day of randomization and ended when the patient obtained a complete or partial hematologic remission, died, or was discharged from the isolation unit.

The isolation unit used in this study consists of a normal bed enclosed in a plastic tent (4). The area available for the patient is about 5 m^2; in this unit circulating air is pumped vertically through high-efficiency filters which are positioned on the ceiling. All procedures on the patient are done through plastic gloves on the sides of the tent. All items entering the unit are either gas- or steam-sterilized. They are passed in and out of the unit through locks irradiated by ultraviolet light. Sterilization of the tent prior to and during its use is performed by cleaning with 2% peracetic acid. The tent is only opened when absolutely necessary. Roentgenograms are performed through the plastic curtain. Any person approaching the isolated patients must wear sterile boots, gloves, gown, and mask. All patients are fed with specially prepared sterile food. Bacteriologic control samples of the isolated area and of the food are taken regularly (twice weekly). Axillary and inguinal areas are rubbed once a day with a polymyxin-neomycin-nystatin ointment. Patients are bathed completely and daily with a 3% hexachlorophene soap. After each bowel movement the buttocks and perineum are washed with 3% hexachlorophene. Nasal ointment (polymyxin-neomycin-nystatin) and throat and mouth wash (polymyxin-neomycin-nystatin) are regularly used after every meal. Fingernails and toenails are cut regularly and cleaned with hexachlorophene soap.

Oral antibiotic regimen.—The decontaminating regimen employed consisted of vancomycin (500 mg), polymyxin (50 mg), and kanamycin (500 mg); the antibiotics were given three times daily as liquid preparations after meals. Nystatin (12×10^6 units/day) was given orally three times a day as 500,000-unit tablets. Gentamicin (480 mg, three times daily) was given to patients who had gram-negative microorganisms cultured from the stools in spite of the administration of the vancomycin-polymyxin-kanamycin regimen. All patients treated with oral nonabsorbable antibiotics outside the isolation unit were kept in single rooms during the entire hospitalization period. The patients left the rooms only for special examinations, such as radiologic or isotopic investigations. They received sterile food and liquid. All patients included in this series whether isolated or not were kept in a special section and were cared for by the same medical and nursing team.

TABLE 1.—Patient diagnoses

Diagnosis	Group 1 (isolation and decontamination; 24 patients)	Group 2 (decontamination only; 21 patients)
Acute myeloblastic leukemia	17	13
Acute lymphoblastic leukemia	2	2
Acute monoblastic leukemia	1	0
Acute promyelocytic leukemia	2	2
Erythroleukemia	0	1
Agranulocytosis	0	1
Lymphosarcoma in blastic transformation	2	2
Total	24	21

Followup cultures.—Routine quantitative aerobic, anaerobic, and fungal cultures of the stools were performed at least once a week during the course of the study. Similarly, cultures were obtained weekly from the nose, gingiva, throat, ear, and axilla. Other bacteriologic studies were performed as indicated clinically.

Fever.—Fever was defined as a rectal temperature > 38°C which persisted for > 6 hours and which could not be attributed to transfusion reactions. Days with infection were defined as days spent with fever associated with microbiologically or clinically documented infections. Patients with fever (> 38°C) and granulocytopenia (< 1000 wbcs/mm^3) were treated with a combination of antibiotics (carbenicillin plus gentamicin, cephalothin plus carbenicillin, or carbenicillin plus cephalothin plus gentamicin) which was started immediately after three blood cultures had been taken. The mean duration of one course of antimicrobial therapy with antibiotics was 5 ± 1.6 days.

RESULTS

The specific diagnoses of the patients included in this study are shown in table 1; 71% of the patients in group 1 had acute myeloblastic leukemia, whereas the proportion with that disease in the group receiving decontamination alone was 65%. The characteristics of the patient population studied were generally comparable, as shown in table 2. The sex and age distribution in the two groups as well as the duration of hospitalization and decontamination were similar. The patients in group 1, however, spent more days in the study with severe neutropenia (defined as a neutrophil count < 100/mm^3).

Antileukemic chemotherapy and general supportive measures during the study were similar; how-

TABLE 2.—Patient characteristics

Characteristic	Group 1	Group 2
No. of patients	24	21
Age range (yrs)	17–72	13–74
Mean age (yrs)	44.8	46.4
Males/females	16/8	14/7
Mean duration of hospitalization ± SD (days)	41.8 ± 19.6	41.4 ± 20.3
Mean duration of decontamination ± SD (days)	34.5 ± 18.6	28.9 ± 15.7
Mean duration in LAFR ± SD (days)	29.4 ± 15.5	0
Mean No. of days (± SD) neutrophil count was—		
$< 1000/mm^3$	33.7 ± 19.1	32.2 ± 17.8
$< 100/mm^3$	27.3 ± 16.0	15.9 ± 8.9

ever, in group 1, cyclophosphamide and cytosine arabinoside were more often employed and at higher doses. This may explain the more severe granulocytopenia in that group. There is no clear reason for the more aggressive chemotherapy in group 1. Since chemotherapy was not uniform in this study, it is conceivable that some patients received more antileukemic drugs because they were isolated. The 24 patients studied in the LAFR remained within the unit for a median of 29 days; the longest time spent in the LAFR was 69 days. The total patient experience was 705 days. Eleven patients tolerated this confinement very well. One of the patients was discharged for psychiatric reasons (schizophrenia) after 59 days in the LAFR. In addition, ten patients, mostly older patients who were not very cooperative, were removed for poor psychologic tolerance of confinement after an average confinement period of 18.6 days (range, 15–23 days). No systematic psychiatric or psychologic support was provided to the patients in this series; this might explain why many patients left the isolation.

The mean duration of oral administration of antibiotics was 34 days in group 1 and 29 days in group 2. The tolerance of the nonabsorbable antibiotic regimen (vancomycin-polymyxin-kanamycin) and nystatin is shown in table 3. Gentamicin at a dose of 450 mg/day was given to four patients in group 1 and to three patients in group 2 after a mean period of 22 days in group 1 and 23 days in group 2. These seven patients had developed micro-organisms (*Escherichia coli*, *Klebsiella* sp., *Streptococcus faecalis*, and *Proteus mirabilis*) which were resistant to the initially administered regimen.

The patients in both groups had a marked reduction of their total fecal microbial flora. Consistent suppression of bacterial growth from stool cultures

TABLE 3.—Patient tolerance to the nonabsorbable antibiotic regimen

Toxicity grade*	No. of patients	
	Group 1 (24 patients)	Group 2 (21 patients)
0	3	4
1	13	11
2	4	3
3	4	3

*0 = none, 1 = mild nausea and vomiting probably not related to antibiotics, 2 = persistent nausea and vomiting related to antibiotics, and 3 = medication modified or discontinued because of nausea or diarrhea.

throughout the duration of the treatment was achieved in 17 patients in group 1 and in 13 patients in group 2. These results and the micro-organisms found in the stool cultures are shown in tables 4 and 5. In 80% of the patients studied a consistent suppression of fungal growth was achieved.

No difference in the sites of infection was observed between the patients in group 1 and those in group 2. The micro-organisms responsible for these infections represented a wide spectrum; 50% were gram-negative bacilli as shown in table 6. In addition, we observed fungal infections in five patients in group 1, two of which were fatal; four patients had pulmonary aspergillosis and one had cellulitis from which *Aspergillus* was cultured. In the group of patients undergoing decontamination only we found three fungal infections. Two were cases of *Aspergillus* infection of the lungs and one was a case of disseminated *Candida* infection, with positive blood cultures, which resulted in death. Other fatal infections were found mostly in patients with bacteremia, which was caused in 80% of the cases by gram-negative rods, or in patients with gram-negative infections of the respiratory tract.

As shown in table 7, there was a statistically significant difference ($P < 0.05$) between the two groups in the number of days with infection as well as in the number of days of treatment with systemic antibiotics. Patients receiving oral antibiotics with-

TABLE 4.—Results of stool cultures

Results	No. of patients*	
	Group 1 (24 patients)	Group 2 (21 patients)
No growth in any of the cultures taken	17 (11)	13 (10)
Microbial growth in ≥ 1 culture	7†	8†

*Nos. in parentheses = mean No. of cultures per patient.
†Micro-organisms are shown in table 5.

TABLE 5.—Micro-organisms found in stool cultures during therapy

Micro-organism	No. of positive stool cultures	
	Group 1 (24 patients)	Group 2 (21 patients)
Escherichia coli	4	5
Candida albicans	3	3
Klebsiella sp.	2	3
Streptococcus faecalis	1	2
Proteus mirabilis	0	1
Serratia marcescens	1	0

out isolation (group 2) had more days with infection and were treated more often with antibiotics than patients who were isolated and received oral antibiotics as well (group 1). It is recognized that the use of such criteria as numbers of days of infection and numbers of days with systemic antibiotics are rather subjective and offer the possibility of bias in interpretation. However, in this study, there was also a significant difference ($P < 0.05$) between the two groups studied in the number of microbiologically proven infections observed. In the patients with granulocyte counts < 100/mm^3, infections were more frequent in the group that received oral antibiotics alone (group 2). It is recognized however, that no statistically significant differences could be found with respect to the incidence of septicemia and fatal infections in this limited study. Moreover, the incidence of infections caused by *Aspergillus* sp., which are presumably airborne, was not reduced in the isolated patients. The responses to cytostatic therapy, ie, the rates of remission, were similar in the two groups. These results are presented in table 8.

TABLE 6.—Type of infection and nature of offending micro-organisms

	No. of infections in patients with a neutrophil count < 100/mm^3	
	Group 1 (24 patients)	Group 2 (21 patients)
Mean No. of days spent with polymorphonuclear neutrophil count < 100/mm^3	621	336
Site or type of infection		
Septicemia	4	4
Lower respiratory tract	4	4
Pharynx	2	2
Urinary tract	2	1
Anus and rectum	1	1
Skin	5	3
Total infections (excluding septicemia)	14	11
Micro-organisms		
Staphylococcus pyogenes *	2	1
Staphylococcus albus *	1	2
Streptococcus hemolyticus A	0	1
Klebsiella pneumoniae *	3	1
Pseudomonas aeruginosa *	2	1
Proteus mirabilis	2	1
Escherichia coli *	2	2
Candida albicans *	0	1
Aspergillus sp.	5	2

* Micro-organisms responsible for positive blood cultures.

DISCUSSION

Infections in patients with leukemia and bone marrow dysfunction occur chiefly during periods of severe granulocytopenia. The infections are usually limited to a few sites and are often caused by organisms acquired within the hospital (6). It is generally agreed that patients who are treated within a protected environment and who are given a prophylactic antimicrobial regimen with nonabsorbable antibiotics have a lower incidence of infections than patients treated under standard ward conditions (1,3,4,8).

Until now, conflicting opinions have been presented as to the efficacy of isolation combined with gastrointestinal decontamination versus gastrointestinal decontamination alone. Previous controlled trials found no advantage for oral nonabsorbable antibiotics used alone as compared to ward care (3,5). On the other hand, the nonramdomized study of Preisler et al (8) showed that nonabsorbable antibiotics could reduce the frequency of infection in neutropenic patients, and another trial (9), utilizing historical controls, noticed a marked reduction in the incidence of severe infection with prophylactic oral nonabsorbable antibiotics. In addition, in three other controlled studies, some advantage from oral nonabsorbable antibiotics over standard ward care was observed as well (4,6,10). Our study also suggests that isolation might be associated with some reduction in the episodes of infection in patients who are also treated with gastrointestinal decontamination as compared to those receiving oral nonabsorbable antibiotics alone. Surprisingly enough, the incidence of airborne infections such as pulmonary aspergillosis was not reduced in our patients. The reduction of bacterial infections in the isolated patients might have been the consequence of a decreased acquisition by these patients of nosocomial pathogens. The better results observed here with respect to cultures of the stools among the group 1 patients are consistent with this hypothesis.

TABLE 7.—Clinical results in severely granulocytopenic patients (polymorphonuclear neutrophil count < 100/mm³)*

	Group 1 (24 patients)	Group 2 (21 patients)	*P* value
Mean total days in study	621	336	
Mean No. of days with infection	365 (588)	147 (438)	< 0.05
Mean No. of days with systemic antibiotics	250 (403)	178 (530)	< 0.05
No. of fungal infections	5 (8)	3 (9)	
No. of fatal infections	7 (11.0)	5 (15.0)	
No. of cases of septicemia (bacterial)	4 (6.0)	4 (12.0)†	
Total No. of infections (excluding septicemia)	15 (24.0)	13 (39.0)	< 0.05

*Nos. in parentheses = No. of days per 1000 days with granulocytopenia (polymorphonuclear neutrophil count < 100/mm³).

†Plus 1 fatal case of septicemia caused by *Candida albicans*.

TABLE 8.—Results of therapy for acute leukemia*

	Group 1		Group 2†	
Results of therapy	Leukemia treated for the 1st time	Leukemia treated after 1 previous remission	Leukemia treated for the 1st time	Leukemia treated after 1 previous remission
Complete remission	4 (10.6)	1 (5.0)	3 (8.8)	0
Partial remission	4 (6.2)	2 (3.8)	6 (6.1)	2 (2.8)
Death	7‡	6‡	5§	4§
Total No. of patients	15	9	14	6

*Nos. in parentheses = mean durations of remission, expressed in mos.

†Group 2 included 1 patient with agranulocytosis who is not considered in this table.

‡Pericardial effusion (1 case), acute pulmonary edema (1), hemorrhage (5), bacterial infection (5), and fungal infection (2).

§Bacterial infection (4 cases), fungal infection (1), and hemorrhage (6).

In eight patients we observed colonization by micro-organisms resistant to vancomycin, polymyxin, and kanamycin which were used for the decontamination procedure, but we have not encountered the emergence of organisms resistant to gentamicin; however, such an occurrence in patients receiving oral gentamicin has already been reported (4,6). It may be speculated that the danger of spreading resistant strains throughout the hospital in patients receiving oral nonabsorbable antibiotics might be limited by strict isolation.

Another major issue in the use of protected environment for prevention of infection in neutropenic patients relates to the rate of complete remission achievable in patients supported by this therapeutic tool in comparison to standard supportive therapy. Schimpff et al (6) observed a significant increase in the number of complete remissions and an increased median survival in patients kept in LAFRs and given oral nonabsorbable antibiotics as compared with a group treated in a normal ward. Bodey et al (1) noted identical remission rates but longer survivals and longer durations of remission in patients who were supported by isolation and oral nonabsorbable antibiotics. These results were presumably due to the more intensive chemotherapy given to the isolated patients. Similar data were published more recently by Rodriguez et al (11).

In controlled studies (3,5) other investigators did not observe more remissions among leukemic patients who were treated in an isolated environment and/or with oral antibiotics as compared to patients treated in regular wards. Our study also failed to show a different response to chemotherapy in the two groups studied, in spite of the fact that more intensive chemotherapy was given to the isolated

patients. In the isolated and decontaminated patients, the frequency of bacterial infection was reduced despite more aggressive therapy and more prolonged neutropenia, but the course of the leukemia was unchanged.

Like many other studies in this field, the present series is small and lacks uniformity in patient populations since it includes a variety of diagnoses and a variety of therapies. However, this investigation was limited to the periods spent with severe neutropenia which is the major factor predisposing to infections in the type of patients studied here. By correcting the incidence of infection for duration of neutropenia we were able to show that LAFRs do reduce the frequency of infection. Isolation, in addition to gastrointestinal decontamination, might thus be beneficial to granulocytopenic patients. However, if the granulocytopenia is caused by leukemia or its therapy, it appears unlikely that this type of supportive care will modify the course of that disease itself since the presently available chemotherapy is unable to maintain prolonged remissions.

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Combination Chemotherapy for Acute Myelocytic Leukemia During Pregnancy: Three Case Reports [1,2]

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SUMMARY

The cases of three patients with acute myelocytic leukemia presenting during the second trimester of pregnancy are summarized. All three patients were treated with combination chemotherapy. One patient, while in remission, underwent elective abortion of an apparently normal fetus. This patient is alive and free of disease 2 years after bone marrow transplantation. Each of the other two patients was spontaneously delivered of a premature infant. Transient bone marrow suppression occurred in one of these infants. Both children were small for their gestational age. One patient died 9 days after her infant was delivered and the other survived for 13 months.

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CASE REPORTS

Patient 1

A 37-year-old white female was in the 32nd week of pregnancy when acute myelocytic leukemia was diagnosed. She presented with a history of excessive bleeding after a tooth extraction, easy bruisability, and recurrent epistaxis. A cbc revealed a hematocrit value of 15%, a leukocyte count of 4200/mm^3, and a platelet count of 11,000/mm^3. A bone marrow aspirate showed almost complete replacement of normal elements by myeloblasts.

Initial therapy consisted of prednisone alone at a dose of 20 mg given orally twice a day × 7 days, but peripheral blood cell counts continued to deteriorate. Combination chemotherapy, consisting of 1 mg of vincristine (VCR) given iv, 2.5 mg of methotrexate and 50 mg of 6-mercaptopurine given orally twice a day, and 60 mg/day of prednisone given orally, was then instituted. Three days later, the patient had premature rupture of membranes and precipitously was delivered of a viable female infant of approximately 34 weeks' gestation. Postpartum complications included gram-negative septicemia. The patient's subsequent course was characterized by several ephemeral remissions, the longest lasting for 6 months. The patient died 13 months after diagnosis from septicemia.

The infant had Apgar scores of 8 and 9 at 1 and 5 minutes after birth, respectively. At birth, the infant had a weight of 2350 g, a height of 45.7 cm, and a head circumference of 30.5 cm. A cushingoid appearance was noted. Peripheral blood cell counts revealed a hemoglobin value of 17.4 g/dl, a hematocrit value of 54.3%, and a leukocyte count of 17,500/mm^3 (this leukocyte count is within the normal range for a premature newborn) (1). Eight weeks later, the infant's weight and height were normal for her gestational age. She was subsequently lost to followup evaluation.

Patient 2

A 22-year-old white female presented in the 17th week of her first pregnancy with a 2-month history

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of malaise and a 2-week history of gingival hyperplasia, generalized lymphadenopathy, and marked hepatosplenomegaly. The hematocrit value was 21%, the leukocyte count was 256,000/mm^3 (99% blast cells), and the platelet count was 4000/mm^3. A bone marrow aspirate was markedly hypercellular with sheets of myeloblasts.

After an initial dose of 8 g of hydroxyurea iv, induction chemotherapy was given as follows: daunorubicin at a dose of 70 mg/m^2/day × 3 days iv; cytosine arabinoside (Ara-C) at a dose of 90 mg/m^2 every 12 hours × 7 days iv; VCR at a dose of 1.0 mg/m^2 on Days 1 and 7 iv; 6-thioguanine (6-TG) at a dose of 90 mg/m^2 every 12 hours × 7 days orally; and prednisone at a dose of 40 mg/m^2/day × 7 days orally. In addition, the patient received cephalothin, gentamicin, and carbenicillin and random-donor packed rbc and platelet transfusions. A subsequent bone marrow aspirate revealed < 5% blast cells.

Four weeks after the diagnosis of acute leukemia was made, the patient underwent an elective abortion without complications. Labor was induced with saline and prostaglandin E_2, and a stillborn male infant was delivered. Marrow function at the time of the abortion was returning to normal with untransfused peripheral blood cell counts as follows: hematocrit reading, 27%; wbc count, 1900/mm^3; absolute neutrophil count, 600/mm^3; and platelet count, 142,000/mm^3. Nine days after the abortion was performed, consolidation therapy was begun with an abbreviated course of the same five-drug regimen. Subsequent monthly maintenance therapy consisted of 6-TG, Ara-C, prednisone, and VCR. Five months after diagnosis, while still in remission, the patient received a bone marrow transplant from her HL–A-matched sister. She remains in complete remission without medication 2 years after transplantation.

The aborted fetus weighed 307.8 g. The estimated gestational age was 124 days by crown-rump measurement (2). No external defects or gross abnormalities in organogenesis were recognized. Fetal organ weights were normal except for the spleen, which was enlarged (0.586 g; normal = 0.35 g) (2). The placenta was unremarkable on gross examination. Possible histopathologic findings were obscured by artifacts caused by saline induction.

Patient 3

A 28-year-old white female in the 27th week of her sixth pregnancy was referred for evaluation of megaloblastic anemia unresponsive to multivitamins and hematinics. Fatigue, recurrent epistaxis, and multiple ecchymoses had been progressive for 2 months prior to admission. Physical findings other than scattered ecchymoses were obscured by massive obesity. An initial cbc revealed a hematocrit reading of 23%, a leukocyte count of 1340/mm^3 (2% blast cells), an absolute neutrophil count of 280/mm^3, and a platelet count of 7400/mm^3. A bone marrow aspirate was hypercellular with 45% myeloblasts and rare megakaryocytes. The patient was treated with the five-drug induction chemotherapy regimen described for patient 2. A bone marrow aspirate performed 6 days after completion of induction therapy revealed 90% blast cells. The following day, a second induction course was begun. The patient received a 7-day course of the same five-drug regimen with doses increased by 30%. An episode of septicemia (*Staphylococcus aureus*) was treated with cefazolin, gentamicin, and carbenicillin. Bilateral pulmonary infiltrates and respiratory failure developed and trimethoprim-sulfamethoxazole and amphotericin B were added to the antibiotic regimen.

Four weeks after admission, the patient spontaneously went into labor and was delivered of a viable male infant. With platelet support, no excessive vaginal bleeding was encountered even though the placenta had to be extracted manually (subsequent histologic evaluation of the placenta was unremarkable). The mother's hematologic status at the time of the delivery included a hematocrit reading of 29%, a leukocyte count of 100/mm^3 (absolute neutrophil count, 0), and a platelet count of 19,000/mm^3. A bone marrow aspirate was markedly hypoplastic. The patient continued to have progressive respiratory failure and died 9 days after childbirth. At autopsy, disseminated *Aspergillus fumigatus* was noted.

The premature infant had Apgar scores of 7 and 8 at 1 and 5 minutes after birth, respectively. At birth, the infant had a weight of 2130 g, a height of 43 cm, and a head circumference of 29 cm. A cbc revealed the following: hematocrit reading, 35%; leukocyte count, 6200/mm^3; absolute neutrophil count, 3200/mm^3; and platelet count, 400,000/mm^3. During the first 2 days the infant was hyponatremic, hyperkalemic, hypocalcemic, and hypoglycemic. The plasma cortisol level was normal. Hematocrit readings ranging between 22% and 25% were noted during the third week of life. The anemia gradually improved and at 7 months the hematocrit reading was 35%. The leukocyte count was 13,700/mm^3 and the platelet count was 200,000/mm^3, which were normal.

During the first 4 months of life, the child had two mild infections: a monilial diaper rash and otitis media. At 13½ months of age, a cbc and general physical examination were unremarkable, but growth parameters were depressed with weight,

height, and head circumference all below the third percentile. Denver Developmental Screening Tests done at 4½ and 13½ months of age were normal.

DISCUSSION

During pregnancy these three leukemic patients were treated with eight different cytotoxic agents. The teratogenic potential of these drugs when given to pregnant laboratory animals has previously been summarized (3). Little data exist regarding the risks to human fetuses; however, first trimester exposure has been associated with a significant incidence of fetal malformations, as well as an increased frequency of early spontaneous abortion (4–6). In contrast, exposure to either single drugs or combination chemotherapy during the second and third trimesters has in general not resulted in gross abnormalities (4,5,7–10). A chromosomal defect, however, has been reported (11). Potential teratogenicity of newer regimens including anthracycline therapy is particularly ill-defined, with published cases thus far reporting only patients in the second or third trimester of pregnancy (12–16).

Fetal effects of cytotoxic agents may be apparent at birth or be delayed for years. Overt congenital malformations and neonatal bone marrow suppression are readily apparent. None of our cases involved a first trimester pregnancy and thus organogenesis was complete. Interestingly, the infant born to patient 3 had relatively normal bone marrow function at a time when the mother's marrow was profoundly hypoplastic. Between the diagnosis of pregnancy and the delivery, mother and fetus were exposed to at least 20 different medications. Retarded growth parameters have, however, been demonstrated during the first 13 months of life. Other postulated long-term effects of in vitro exposure to chemotherapeutic agents include retarded mental development, sterility, and an increased risk of developing a malignancy (5,6,17).

In designing an approach to therapy for the pregnant leukemic patient, consideration must also be given to the risk to the mother posed by the fetus. In an attempt to avoid complications which may be associated with an early, spontaneous delivery, an elective abortion can be considered for those patients in the first or early second trimester. In the relatively stable patient with adequate bone marrow function, abortion prior to institution of chemotherapy may be considered. Most newly diagnosed leukemic patients, however, have severely compromised marrow reserves and an early abortion may further jeopardize their status. This subset of patients, as exemplified by patient 2, is perhaps best managed with chemotherapy in an effort to attain remission before terminating the pregnancy. When leukemia is diagnosed late in the second or third trimester, the evidence suggests that both mother and fetus often tolerate large doses of chemotherapeutic agents without serious complications (7–10,12,13,15,16). Therapy for the mother, therefore, may not need to be compromised by the presence of the fetus. It should be emphasized, however, that continued long-term evaluations of children who have been exposed to cytotoxic drugs in vitro are necessary in order to more clearly define the ultimate risks.

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Combination Chemotherapy In Vitro. III. BCNU [1,2]

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Nitrosourea derivatives are antitumor compounds that have received extensive preclinical and clinical investigation. They offer novel chemical structures, superior activity in experimental tumor systems, and unique modes of antitumor action (1,2). All classic nitrosourea derivatives (BCNU, CCNU, and methyl-CCNU [meCCNU]) have qualitatively similar spectra of antitumor activities (3,4), clinical toxic effects (5), metabolic disposition (6), and degradation (7) and appear to act through similar mechanisms (7,8). However, attachment of a glucose carrier to the cytotoxic group results in compounds such as streptozotocin and chlorozotocin which possess less myelosuppressive toxicity and display alkylating and carbamoylating activities that are quantitatively different from those of classic nitrosoureas (9,10).

BCNU was the first nitrosourea congener with relatively low toxicity and significant antitumor activity to merit clinical trials (1,11). During the past 10 years, BCNU has been combined with two or more antitumor drugs in a variety of regimens designed to improve its anticancer efficacy (1–4). In our laboratory we investigate cellular responses of cultured human cells to the lethal effects of antitumor agents. Currently, we are extending these studies to drug combinations in an attempt to detect synergistic pairs with potential clinical application. A drug is paired with another to determine whether the first drug can increase the killing effect of the second.

This report, the third in a series (12,13), describes results obtained by pairing BCNU with ten other antitumor agents for the in vitro treatment of cultured human lymphoma cells.

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MATERIALS AND METHODS

Monolayer cultures of T_1 cells, a human lymphoma cell line (14), were maintained in Ham's F-10 medium supplemented with 20% fetal calf serum, glutamine, vitamins, and antibiotics. Cell survival was assayed by the colony-formation technique method as previously described (12,13,15). Drug solutions were freshly prepared before each experiment and appropriate dilutions in growth medium were applied in pairs (ie, BCNU and adriamycin, BCNU and bleomycin, etc) to the cells for 1 hour at 37°C (pH 7.0–7.2). BCNU was always used at a concentration of 50 μg/ml which reduces the survival of T_1 cells by $> 80\%$. Other drugs were given in increasing concentrations. In every experiment, control survival curves for each individual drug were obtained independently. In experiments involving adriamycin, cells were incubated with BCNU alone for 1 hour at 37°C. The drug was discarded and the cells were thoroughly washed with medium and then exposed to increasing concentrations of adriamycin for an additional hour. This was done to prevent an artifactual antagonistic effect which was previously noted when these two agents were applied simultaneously (12).

Data were analyzed with a digital computer and predicted additive effects were compared to the observed response. Each combination experiment was performed at least twice with three replicates per dose point and six replicate controls (untreated cells). Significant deviations from the additive response were demonstrated as previously described (12,13). The magnitude of these deviations was quantified by a performance index. The performance index was obtained by averaging the sum of ratios of observed and expected survivals for each dose point. To calculate the ratios of synergistic drugs, the observed response was used as the denominator. The expected response was used as the denominator in antagonistic situations. This was done in order to obtain values > 1, so that the efficiency of antagonistic and synergistic drugs could be compared.

RESULTS

With the exception of *cis*-acid, a water-soluble

nitrosourea derivative (16) which showed subadditive killing, every agent used in combination with BCNU showed supra-additive or true synergistic effects (table 1). Synergism was elicited with bleomycin, adriamycin, *cis*-dichlorodiammineplatinum(II) (DDP), VP-16-213, and two nitrosourea congeners, meCCNU and CCNU. The performance index of the BCNU-adriamycin combination was twice that previously obtained with the adriamycin-BCNU pair (12), while that elicited with DDP was only 60% of that obtained for the reversed pair (11). The most significant synergistic activity was observed for combinations of BCNU with bleomycin, adriamycin, DDP, and meCCNU.

TABLE 1.—Lethal effects of simultaneous treatment with BCNU and other drugs on human lymphoma cells*

Drug	Survival of T_1 cells treated with BCNU alone (mean ± SE)	Concentration (μg/ml)	% survival with drug alone (mean ± SE)	% expected survival in combination (additive effect)	% observed survival in combination (mean ± SE)	Significant deviation from predicted response†	Performance index
Bleomycin	16.0 ± 0.82	10	31.0 ± 3.6	4.95	3.6 ± 0.53	S ($P < 0.01$)	7.07
		25	15.2 ± 0.72	2.5	1.5 ± 0.08		
		50	4.4 ± 0.24	0.70	0.42 ± 0.04		
		100	3.8 ± 0.83	0.61	0.03 ± 0.01		
Adriamycin	13.4 ± 3.06	0.05	62.0 ± 5.9	8.31	5.4 ± 0.51	S ($P < 0.01$)	7.24
		0.1	60.3 ± 3.8	8.04	1.1 ± 0.3		
		0.25	39.0 ± 6.2	5.2	0.38 ± 0.07		
		0.5	13.0 ± 1.9	1.7	1.7 ± 0.4		
DDP	5.23 ± 0.7	0.5	95.6 ± 4.4	4.9	7.4 ± 0.54	S ($P < 0.01$)	5.35
		1.0	93.0 ± 2.2	4.8	4.3 ± 0.38		
		2.5	86.0 ± 8.6	4.5	3.0 ± 0.16		
		5.0	26.3 ± 1.3	1.4	0.09 ± 0.02		
		7.5	15.2 ± 1.5	0.8	0.09 ± 0.01		
MeCCNU	5.4 ± 0.69	10	99.0 ± 4.8	5.32	4.1 ± 0.5	S ($P < 0.01$)	22.9
		25	92.3 ± 6.0	4.97	1.3 ± 0.04		
		50	20.0 ± 0.61	1.06	0.1 ± 0.03		
		75	2.1 ± 0.06	0.11	—		
CCNU	7.8 ± 0.8	10	100.0 ± 8.8	7.8	2.42 ± 0.12	S ($P < 0.01$)	2.38
		25	58.8 ± 3.0	4.58	0.82 ± 0.12		
		50	6.0 ± 0.9	0.47	0.09 ± 0.007		
		75	1.5 ± 0.15	0.12	—		
cis-Acid	16.7 ± 1.1	10	86.8 ± 6.2	14.5	21.1 ± 2.2	A ($P = 0.222$)	1.34
		25	48.8 ± 3.0	8.1	9.4 ± 0.6		
		50	10.2 ± 0.6	1.71	2.3 ± 0.9		
		75	4.3 ± 0.1	0.72	1.0 ± 0.4		
Yoshi 864	6.6 ± 1.3	10	61.0 ± 3.5	11.4	17.1 ± 2.0	S ($P < 0.01$)	1.27
		25	53.0 ± 4.4	9.9	9.0 ± 0.58		
		50	35.2 ± 2.3	6.6	5.3 ± 0.42		
		75	26.0 ± 1.6	4.8	2.3 ± 0.18		
		100	18.0 ± 0.92	1.2	—		
Camptothecin	19.1 ± 2.0	5	89.1 ± 9.3	17.0	14.5 ± 2.4	S ($P <= 0.03$)	1.08
		10	82.4 ± 6.3	16.1	12.1 ± 1.0		
		25	41.2 ± 1.6	8.3	16.2 ± 1.8		
		50	52.7 ± 3.5	10.1	7.0 ± 0.86		
		75	37.6 ± 1.5	7.2	7.7 ± 0.72		
Prednisolone	10.4 ± 0.52	10	73.9 ± 11.5	7.7	3.5 ± 0.3	S ($P < 0.01$)	2.29
		25	89.9 ± 2.3	9.4	7.1 ± 1.4		
		50	86.5 ± 6.6	9.0	5.2 ± 0.57		
		75	82.3 ± 2.0	8.6	2.2 ± 0.14		
		100	73.2 ± 3.0	7.6	3.3 ± 0.43		
VP-16-213	13.0 ± 1.9	1	53.3 ± 5.2	6.9	5.2 ± 0.4	S ($P < 0.01$)	3.96
		2.5	36.3 ± 5.0	4.7	0.7 ± 0.1		
		5.0	13.7 ± 1.3	1.8	0.15 ± 0.06		

*Results are from a single representative experiment for each combination.
†S = synergistic; A = antagonistic.

DISCUSSION

Sequential treatment of T_1 cells with BCNU followed by exposure to increasing concentrations of adriamycin resulted in a marked synergistic killing effect (performance index, 7.24). These results are similar to those previously reported for the sequence of adriamycin followed by escalating doses of BCNU (12), with the exception that the performance index of the present sequence was twofold greater than that obtained with the previous pairing. Conversely, the performance index of the BCNU-DDP pair applied simultaneously was only 60% that of the DDP-BCNU pair, where a single concentration of DDP was paired with increasing doses of BCNU. It is possible that such results are due to different ratios of the drugs used and that synergistic effects and different performance indices can be appreciated by pairing drugs in multiple ratios (17). Another possibility is that small biologic variations seen at low survival points in experiments separated in time may yield different cell survival values for a given ratio of drugs; these in turn may influence considerably the value of the performance index. For instance, in experiments conducted > 1 year earlier, a concentration of 50 μg/ml of BCNU plus 10 μg/ml of DDP reduced cell survival to 0.3%; in the present set of experiments 50 μg/ml of BCNU plus 7 μg/ml of DDP decreased survival to 0.09%.

A remarkable synergistic effect was noted for bleomycin, although the mechanism is not clear. Since these two agents have different clinical toxic effects, the BCNU-bleomycin pair may represent a clinically exploitable combination which could result in a significant gain in tumor cell killing levels.

The highest performance index was for the BCNU-meCCNU pair, while the only subadditive results occurred with the combination of BCNU and *cis*-acid. These results again suggest differences in mechanisms of action for nitrosourea derivatives (5,18–21).[4]

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Effects of CCNU on Hematopoiesis in Rats [1,2]

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SUMMARY

The effect of orally administered CCNU on proliferating and cytokinetically "resting" bone marrow cells was studied in rats. The resting bone marrow cells were selectively labeled with ^{3}H-thymidine by the "complete ^{3}H-thymidine labeling method." The results show a reduction in the number of absolute bone marrow cells to about 40% of the original value in 3 days. Regeneration was completed only after 22 days. No difference was found in the destructive or the regenerative pattern of CFU-C, myelopoietic, erythropoietic, lymphatic, or megakaryocytic cells. Resting and proliferating cells of the bone marrow were equally affected by CCNU.

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Compounds of the nitrosourea group have been found to be effective against extracerebral tumors such as leukemias, carcinomas, and sarcomas (1–4). These substances are lipid-soluble and capable of crossing the blood-brain barrier (5), and therefore are also useful against intracranial tumors in man (6). Some observations indicate that they are cell cycle-nonspecific agents (7). Toxicity from the use of the agents has been reported, especially in intestinal and hematopoietic tissues (8). In the latter, cumulative toxicity was demonstrated by a decrease in the number of platelets, leukocytes, and erythrocytes, and recovery was not always complete within a period of 4–6 weeks (9–11). Furthermore, a plasma concentration-dependent toxicity for hematopoietic precursor cells (CFU-C) in man and mice from CCNU and the *cis*- and *trans*-2-hydroxylated metabolites could be demonstrated (12,13).

In mammalian bone marrow, the committed hematopoietic precursor cells (CFU-C) and the hematopoietic cells have a very rapid turnover, whereas the uncommitted pluripotent stem cells (ie, the cells capable of repopulating an aplastic bone marrow) have a very slow reproductive rate under physiologic steady-state conditions (14).

Since there is no information available on the effect of the compounds of the nitrosourea group on these cytokinetically "resting" bone marrow cells, a selective labeling of "resting bone marrow cells" by means of tritiated thymidine (^{3}H-TdR) (15) seems to be particularly useful for the study of CCNU effects on slowly as well as rapidly proliferating bone marrow cells. This labeling method was used previously in rats by our group of investigators (14–16). The effect of CCNU was therefore investigated in rat bone marrow, with special emphasis on the regenerative processes.

MATERIALS AND METHODS

The experiments were carried out with the offspring of Wistar rats which had been continuously infused with ^{3}H-TdR [5] from Day 20 of pregnancy until delivery (16). The amount of ^{3}H-TdR administered per day was 2.1 microcuries/g of body weight. The specific activity of ^{3}H-TdR was 2.0 curies/mmol. The 100% ^{3}H-TdR-labeled newborn rats were subsequently injected every 12 hours sc with ^{3}H-TdR (specific activity, 2.0 curies/mmol) at a dose level of 0.5 microcurie/g/day × 6 weeks after birth. Four

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[5]^{3}H-TdR was obtained from the Radiochemical Centre, Amersham Searle Co, United Kingdom.

weeks after discontinuation of ^{3}H-TdR (10 weeks after birth), 46 young animals from 13 mothers were divided into a control group of 22 animals and an experimental group of 24 animals. The control animals received no further treatment; seven animals were killed at the beginning of the experiment and groups of three were killed 2, 12, 19, 22, and 26 days later. The animals of the experimental group received a single dose of 100 mg of CCNU/kg of body weight orally and were killed 12 hours or 1, 2, 3, 12, 19, 26, or 30 days later. Thirty-six unlabeled 10-week-old rats received a single dose of 100 mg of CCNU/kg orally and were killed 12 hours and 1, 2, 3, 4, 5, 9, 14, 18, 22, 26, and 30 days later. At each investigation, three animals were killed. The right femur and tibia were used for bone marrow smears. They were stained with May-Grünwald-Giemsa. The bone marrow of the left femur between the epiphyses was blown out, weighed, and then suspended in 5 ml of a solution of one part 1.104% Na_2EDTA in 7% saline and four parts 0.9% saline. The number of nucleated cells was determined in a hemocytometer. The results were expressed as nucleated cells per milligram of bone marrow. The number of megakaryocytes was determined in the smears by evaluating the cells/mm^2.

Granulopoietic progenitor cells (CFU-C) in the bone marrow of ten control animals and of 15 CCNU-treated 10-week-old animals were assayed by an in vitro culture as previously described (17). Autoradiographs of bone marrow smears from labeled animals were prepared with Kodak AR 10 stripping film exposed for 45 days. After photographic processing, the smears were stained with Giemsa at pH 5.75. All autoradiographs, including controls, were processed at the same time. Bone marrow smears were evaluated as follows: 500 bone marrow cells in each preparation were differentiated. In the autoradiographs the number of labeled cells per 1000 bone marrow cells was counted and differentiated into lymphocytes and matrix cells. The mean grain count per labeled cell was determined from 200 labeled cells of each cell class. Autoradiographs from unlabeled smears showed that the background was < 5 grains/cell at the given exposure time. Labeled cells were therefore considered to be cells with ≥ 5 grains.

RESULTS

Bone Marrow of the Control Group

The labeling procedure used in this experiment resulted in a complete labeling of all nucleated bone marrow cells immediately after the last ^{3}H-TdR injection when the animals were 6 weeks old. Four weeks later, at the beginning of the CCNU experiment, all proliferating cells had lost their labeling by a decrease of labeling intensity beyond the background level. Only slowly proliferating cells of the bone marrow were found to be still labeled. These comprised cells of the bone marrow matrix, ie, reticulum or endothelial cells, and a small fraction of labeled bone marrow lymphocytes. The total number of nucleated cells per milligram of bone marrow amounted to 1.6×10^6 cells. The total number of labeled cells was calculated as 79100/mg of bone marrow. Forty-nine percent of all labeled cells were matrix cells and 51% were labeled lymphocytes.

TABLE 1.—No. of nucleated cells in bone marrow 72 hrs after CCNU administration

Cell class	No. of nucleated cells $\times 10^3$	
	72 hrs after CCNU (3 animals)	Control (7 animals)
Nucleated erythropoietic cells	98 (± 13.7)*	636 (± 113.2)
Myelopoietic cells	222 (± 22.9)	382 (± 34.6)
Lymphocytes	251 (± 29.4)	910 (± 88.9)
Matrix cells	5 (± 0.7)	38 (± 4.8)
Blast cells	52 (± 5.6)	139 (± 16.9)
Total	790	2749

*Nos. in parentheses = ± 1 SE of the mean.

Effect of CCNU on the Number of Bone Marrow Cells

As shown in figure 1, the number of nucleated cells per milligram of bone marrow was markedly reduced by the CCNU treatment. There is a steady decline of the cell number from the original value of 1.6×10^6 cells to 40% of this value between 48 and 96 hours after CCNU administration. Delayed regeneration was observed, with normal values attained 22 days after CCNU application. The mean numbers of nucleated cells $\times 10^3$ in the bone marrow at the time of the severest destruction, ie, 72 hours after CCNU administration, are indicated in table 1.

Regeneration did not start until the fifth day after the beginning of the experiment. Normal values were not attained until the 22nd day. The number of megakaryocytes in bone marrow smears was 100/mm^2 at the beginning of the experiment.

TABLE 2.—CFU-C values after CCNU application

	No. of CFU-C cells/10^5 nucleated marrow cells	CFU-C cells/mg of bone marrow
Control group	51.5 ± 3.1*	824.0
CCNU administered on Day—		
3	0	0
4	3.6 ± 1.1	24.5
7	31.6 ± 3.0	306.5
16	49.0 ± 2.6	568.4
21	47.3 ± 4.1	756.8

*CFU-C from triplicate samples ± 1 SE of the mean.

Since many karyolytic, pyknotic, multinucleated, and giant cells were induced by CCNU during the first few days after the treatment, an evaluation of megakaryocytes for the first 5 days was not possible. However, after Day 5, there was a steady decline in the number, with minimum levels of 10/mm² at Days 14 and 18. At Day 22, normal values were again observed.

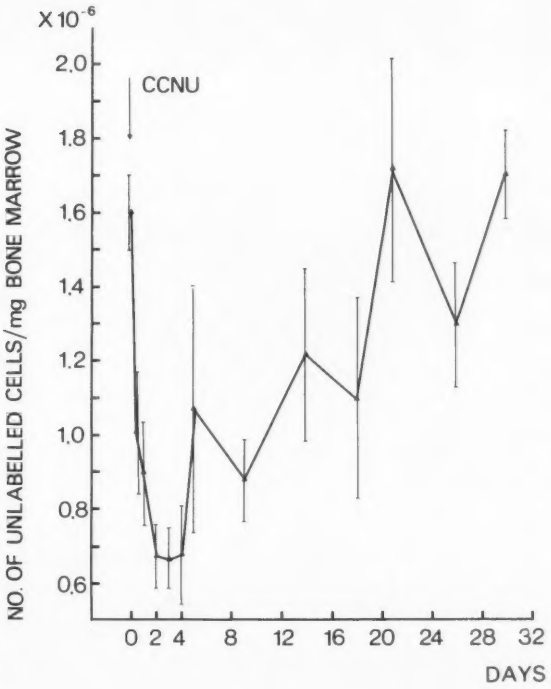

FIGURE 1.—No. of nucleated unlabeled bone marrow cells/mg of bone marrow as a function of time after CCNU administration. Vertical bars = SE. At Day 0 the values are from 7 controls; at all subsequent days the values are from 3 treated rats.

The myelosuppressive activity of CCNU was further confirmed by its effect on granulocytic precursor cells (CFU-C) of rat bone marrow. A maximum depression of CFU-C concentration occurred 3 days after drug treatment and a recovery to baseline values was seen at Day 21 (table 2).

Effect of CCNU on Peripheral Blood Cells

The mean number of neutrophils, lymphocytes, and thrombocytes of the seven control animals killed before the beginning of the experiment and of the 24 experimental animals before treatment with CCNU are indicated in figure 2 at Day 0. Application of CCNU in the 24 experimental animals resulted in profound leukopenia in peripheral blood as shown in figure 2. Minimal values for the number of circulating lymphocytes were found 48 hours after treatment. An increase, suggestive of regeneration, could be observed starting on the 14th day after CCNU application. Labeled lymphocytes were detected in blood smears but their frequency was so low that it was impossible to establish any change in their number or labeling intensity. As also shown in figure 2, there was marked thrombocytopenia with minimum levels observed at 4 and 14 days after CCNU. Thereafter, increasing numbers of platelets were counted. Normal levels were demonstrated on Day 22.

Effect of CCNU on Slowly Proliferating Labeled Bone Marrow Cells

The changes in labeling intensity of physiologic slowly proliferating bone marrow cells after administration of CCNU are shown in figure 3, in which the mean grain count above background per labeled cell is plotted as a function of time. There was an obvious decrease of mean grain count for matrix cells and bone marrow lymphocytes in the experimental group. The decrease of labeling intensity was already demonstrable 12 hours after administration of CCNU. In both cell types, the grain count decreased within 3 days to about one half the original level. Thereafter, a further decrease up to the 12th experimental day to almost background level was noted in the lymphocytes, whereas the matrix cells showed only a slight change in mean grain count. In figure 4, the number of labeled matrix cells and labeled lymphocytes is plotted. The data are based on the evaluation of 1000 nucleated cells/animal and calculated per milligram of bone marrow. The absolute number of labeled cells per milligram of bone marrow in the controls at Day 0 was 38000 for matrix cells and 41000 for lymphocytes. After administration of CCNU, an initial

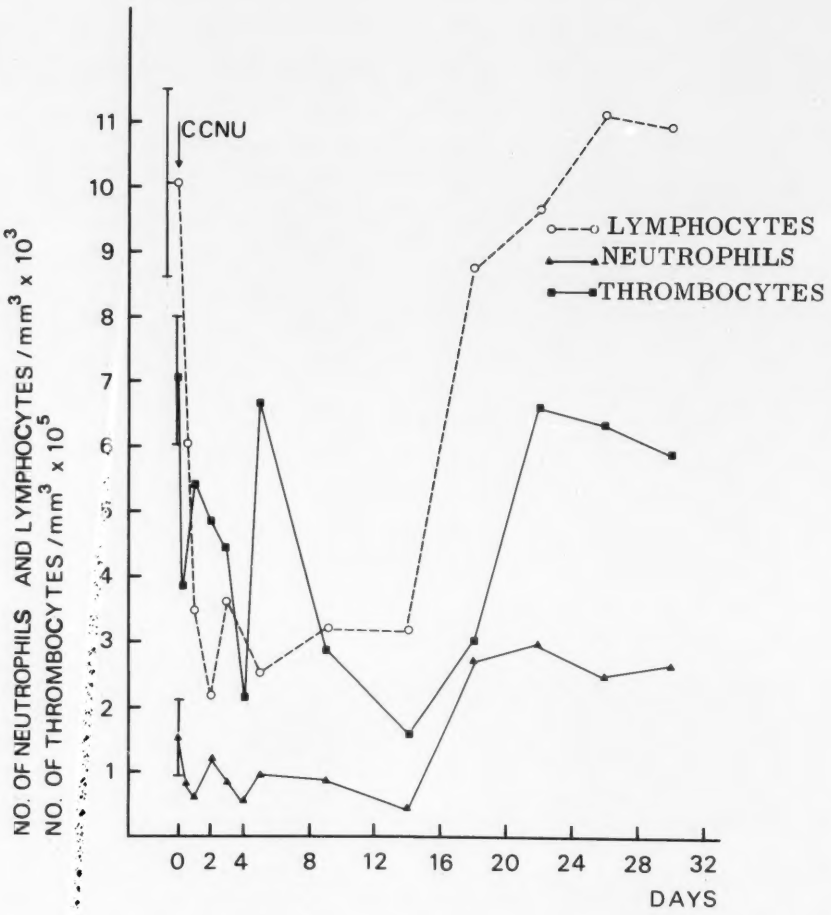

FIGURE 2.—No. of peripheral blood cells as a function of time after CCNU application. Vertical bars = SE. At Day 0 the values are from 7 controls and 24 experimental animals before treatment; at all subsequent days the values are from 3 treated and killed animals.

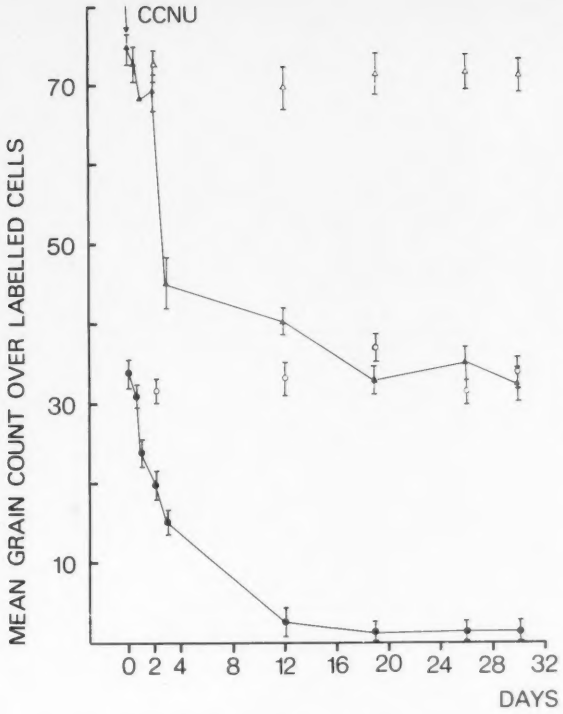

FIGURE 3.—Labeling intensity of resting bone marrow cells as a function of time after CCNU application. Vertical bars = SE. ▲—▲ = matrix cells and ●—● = lymphocytes from CCNU-treated animals; △ = matrix cells and ○ = lymphocytes from CCNU-untreated animals. At Day 0 the values are from 7 animals; at all other time points the values are from 3 animals.

decrease and later an increase of cell numbers on the third day was seen in the experimental group. Between the third and the 12th days after CCNU there was a steep drop in the number of labeled cells. An additional slight decrease of the values was observed in lymphocytes. At the end of the observation time, only small numbers of the labeled bone marrow cells were demonstrable.

DISCUSSION

During the last decade, the nitrosourea derivatives have been found to be effective against a variety of tumors (18). The main toxic effect is a delayed suppression of peripheral blood cells. In particular, marked thrombocytopenia is the dose-limiting factor of these substances. Treatment in man resulted in moderate leukopenia for 20 days and thrombocytopenia for > 30 days after CCNU application. Restoration of platelet counts was demonstrable in some patients only after 125 days (19). CCNU obviously has a cumulative toxicity if more than five cycles are given (19). An early drug metabolism could account for the delayed hematopoietic toxicity of the agent. Studies in man have shown that virtually no CCNU could be detected in the plasma of patients receiving CCNU orally (20). There is some indication from the response of rat brain tumors to CCNU that the nitrosourea derivatives are cell cycle-nonspecific agents (7,21). Specifically, the effect of nitrosoureas was unchanged when the rate of proliferation of hematopoietic precursor cells was increased (7).

The main objective of the present investigation was to study the effects of CCNU on the cytokinetically different cell types in the bone marrow. For this purpose, the experiments were done in rats since, in previously reported experiments (15), the existence of morphologically distinct classes of cytokinetically resting bone marrow cells was demonstrated. This evidence was provided by labeling bone marrow cells of newborn rats with ^{3}H-TdR administered repeatedly over the time of bone marrow development. The cells which retained their ^{3}H-labeling for weeks after the last ^{3}H-TdR administration were identified morphologically as matrix cells and a small fraction of lymphocytes. In comparison, the cells of rapidly proliferating cell systems of rat bone marrow fall below the level of autoradiographic detectability within a few days after the last ^{3}H-TdR administration (15). No radiotoxic effect on these resting cells could be found when the ^{3}H-TdR dose administered did not exceed the dose used in this study (22). Specifically, the number of non-proliferating cells does not alter after stopping the radioactive tritium for 30 weeks (15). Furthermore, the labeling pattern of the resting cells is not affected by re-utilization of labeled DNA breakdown products (23).

From the experiments described here, it was evident that after CCNU administration the total number of bone marrow cells was reduced to about 40% of the original values within 48 hours. Repopulation of the marrow was very slow, with normal cell numbers not attained until 22 days after CCNU administration. The effect was not restricted to the known proliferating cells in the bone marrow, but was also demonstrable in the cytokinetically resting cells. These cells were selectively labeled at the beginning of the experiment so that they would not be reduced in number by cell division during the 4 weeks after discontinuation of the ^{3}H-TdR application.

The changes in the absolute number of labeled bone marrow cells should be compared with the pattern of labeling intensity: during the first 3 days

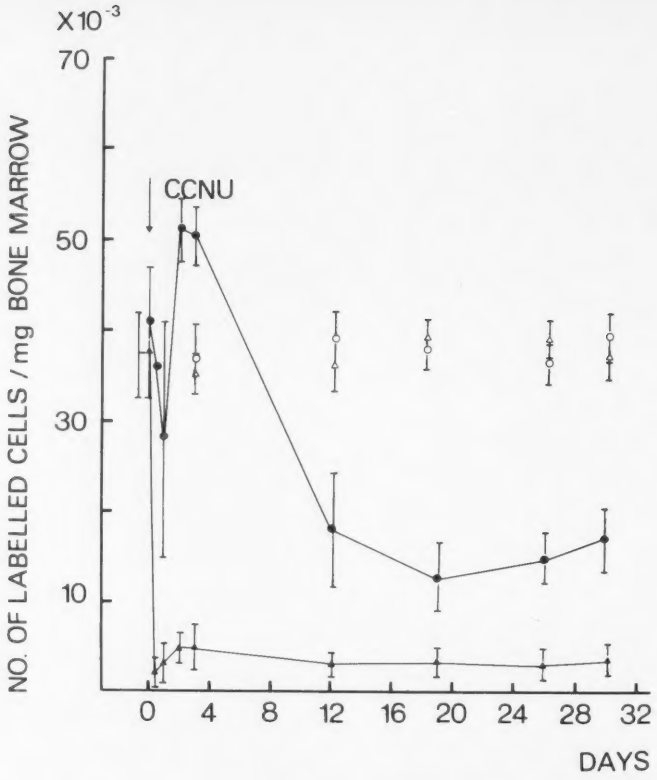

FIGURE 4.—No. of nucleated resting bone marrow cells/mg of bone marrow as a function of time after CCNU administration. Vertical bars = SE. ▲——▲ = matrix cells and ●——● = lymphocytes from CCNU-treated animals; △ = matrix cells and ○ = lymphocytes from CCNU-untreated animals. At Day 0 the values are from 7 animals; at all other time points the values are from 3 animals.

after treatment the labeling intensity of these cells decreased to about 50% of the original values. At the same time, the total number of labeled lymphocytes increased. This behavior can be interpreted as cell division. Later in the experiment, a decline in the number of labeled lymphocytes is apparent, with a further decrease in labeling intensity. The explanation for this finding may be that, after an initial triggering, most of the labeled lymphocytes are destroyed by CCNU. In matrix cells a very early destruction of most of the cells obviously took place, as indicated by the decrease in the cell numbers. However, the remaining cells divided and their grain counts were thereby continuously diluted, so that the labeling of these cells, after several divisions, was no longer distinguishable from background. In conclusion, we were able to show that CCNU has similar effects both on proliferating and on cytokinetically resting bone marrow cells.

As far as the small labeled fraction of lymphocytes is concerned, the findings indicate a stimulation of proliferation as well as destruction. These results are comparable with our earlier investigation of the labeling pattern of this cell class during recovery of bone marrow cellularity after X-irradiation or cytotoxic agent therapy (24–27). Here it was found that these cells play an important role in the recovery of hematopoiesis.

In previous studies (24,25), matrix cells did not change in number or labeling intensity after administration of cytotoxic agents effective on proliferating cells; in contrast, this current study showed that matrix cells were reduced in number by CCNU. They showed proliferative activity, initiating the restoration of the depleted bone marrow.

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Clinical Evaluation of Peptichemio [1,2]

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SUMMARY

Peptichemio is a peptide complex of *m*-L-phenylalanine mustard. A clinical evaluation of this agent was conducted in 116 patients, of whom 104 were evaluable for both toxicity and response. The majority of patients had solid tumors. The drug was administered iv daily for 3 days at doses of 20–75 mg/m²/day, with courses repeated at 3–4-week intervals. The optimal dose schedule appears to be 45 mg/m²/day for 3 days. The major side effects were cumulative myelotoxicity, phlebitis, and mild nausea and vomiting. No other major organ toxicity was observed. The partial remission rate was 7%. Most patients had received an alkylating agent as part of their previous therapy. There were seven partial responses and four less than partial responses achieved in patients with melanoma, lymphoma, and gastrointestinal, genitourinary, breast, and head and neck carcinomas. Responses lasted 4–36 weeks.

[Cancer Treat Rep 63:385–389, 1979]

Peptichemio is a compound composed of synthetic peptides linked by covalent bonds to *m*-[di-(2-chloroethyl)-amino]-L-phenylalanine (*m*-L-phenylalanine mustard). This was one of the many peptide complexes of mustard synthesized by De Barbieri and co-workers (1) with significant antitumor activity. At the molecular level, it inhibits DNA synthesis, DNA polymerase, and reverse transcriptase (2). In addition, it inhibits protein synthesis, probably mediated at the amino acid level of the peptide complexes. Electron microscopic studies have further supported these biochemical findings. Peptichemio increased the survival time of mice and rats bearing L1210 leukemia and Yoshida sarcoma respectively (2). The drug also decreased the tumor weight in treated mice bearing sarcoma 180 compared to controls. Its basic mechanism of action is that of an alkylating agent with activity mediated via the dichloroethylamino group. Some investigators have stressed that all the amino acids in the peptides must belong to the L-configuration for optimal antitumor effect, and that this drug also has antimetabolite activity mediated via the peptide moieties (1–3). These observations have been refuted by others (4), who also did not observe any major differences in the therapeutic indices of peptichemio and other alkylating agents examined in various animal tumor systems. This same group of investigators has reported that L1210 leukemia cell lines resistant to peptichemio remained sensitive to cyclophosphamide and mechlorethamine but showed cross-resistance to melphalan (5).

In vitro and in vivo studies of the antimitotic activity of peptichemio have been carried out in cultured lymphocytes and in animal tumor systems (2,6,7). Morphologic studies confirmed its effect on cell division by inducing mitotic damage and chromosomal aberrations. The effect appeared to be dose-dependent. Patients who received peptichemio as therapy were also noted to have increased chromosomal aberrations shortly after treatment. It is not known whether these changes are permanent. In vitro immunologic studies in lymphocyte cultures have demonstrated that peptichemio is weakly immunosuppressive, especially when compared to the effect of melphalan (2). Also, these studies seemed

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to suggest that peptichemio has a greater inhibitory effect on B lymphocytes.

Clinical evaluation of peptichemio has been conducted in some European centers, with successes reported against a variety of malignancies (8–13). Different doses and schedules have been explored with similar results. A dose schedule of 25 mg/m^2 iv daily for 3–5 days, repeated every 3–4 weeks, was well-tolerated by most patients. We report the results of a study of peptichemio in patients treated at the M. D. Anderson Hospital and Tumor Institute.

MATERIALS AND METHODS

Adult patients with documented metastatic malignant diseases for whom there was no proven effective therapy or patients who were no longer responsive to effective therapy were eligible to receive peptichemio. Patients refractory to therapy with other alkylating agents were not excluded from the study. None of the patients had received chemotherapy or radiotherapy during the preceding 3 weeks. Except for patients with acute leukemia, the initial absolute granulocyte count was $> 1500/mm^3$ and the initial platelet count was $> 100{,}000/mm^3$ in all patients. All but 15 of the patients had a serum bilirubin level of < 2 mg/dl and a serum creatinine level of < 1.5 mg/dl. Informed consent was obtained from all patients prior to therapy, according to institutional policy.

Pretreatment evaluation included a complete history and physical examination with documentation of any measurable disease, an SMA-12 profile, a cbc, and differential and platelet counts. Bone marrow aspiration and other appropriate laboratory and radiologic studies were performed as indicated and were repeated at appropriate intervals. Blood cell counts were obtained once or twice weekly during therapy and an SMA-12 profile was obtained prior to each course of therapy.

A complete remission (CR) was defined as disappearance of all measurable evidence of tumor. A partial remission (PR) was defined as a $\geq 50\%$ reduction in the sum of the product of the diameters of measurable lesions, provided no lesion increased in size and no new lesions developed. Stable disease was defined as a response of $< 50\%$ (less than a PR) or no further increase in previously progressing disease after initiation of therapy. The duration of remission or stable disease was determined from the start of therapy until progressive disease ($> 25\%$ increase in the size of a lesion) became evident. Early death was defined as death occurring within 2 weeks after the initiation of therapy and such patients were excluded from the analysis of response. Two of these 12 patients survived long enough to be included in the analysis of drug toxicity.

Peptichemio was supplied by Dr. A. De Barbieri of the Istituto Sieroterapico Milanese, Milan, Italy, in 0.5-ml ampules containing 40 mg of the peptide complex in 40% propylene glycol as a carrier. It was administered after dilution in 50–100 ml of 5% dextrose solution and was protected from light exposure. The initial starting dose was 20 mg/m^2 iv daily given for 3 days, with each course repeated at approximately 3-week intervals depending on bone marrow recovery. Dose escalations proceeded at 5–10 mg/m^2/day during subsequent courses. In general, approximately three patients were studied at each dose level and were evaluated for at least 3 weeks before additional patients were entered at a higher dose level. The dose of peptichemio administered to individual patients was escalated during subsequent courses if no toxicity was observed during the preceding course. The dose was reduced in any patient who developed unacceptable toxicity.

RESULTS

A total of 116 patients were entered in the study, of whom 104 were evaluable for response and 106 for toxicity. Twelve patients died within 2 weeks and could not be evaluated for response, but two of them survived long enough to manifest drug toxicity. Among the evaluable patients there were 65 males and 41 females, and the median age was 49 years with a range of 17–77 years.

The doses of peptichemio used in this study ranged from 20 to 75 mg/m^2/day for 3 days (table 1). The majority of patients received a dose of 45 mg/m^2/day. A total of 261 courses were administered.

Toxicity

The percent of patients who experienced nonhematologic toxic effects at various dose levels is shown in table 2. The most common side effects encountered were nausea and vomiting, phlebitis,

TABLE 1.—Patients entered at each dose level

Dose (mg/m^2/day × 3 days)	No. of patients treated	No. of courses
20–30	21	24
35–40	37	60
45	65	101
50–55	38	60
60–75	12	16
Total	173	261

TABLE 2.—Nonhematologic toxic effects

Dose (mg/m²/day × 3 days)	No. of patients treated*	% of patients with— Nausea and/or vomiting	Phlebitis	Alopecia	General malaise	Light-headedness	Stomatitis	Urticaria and/or rash	Fever†	Bleeding†
20–30	22‡	18	14	5	5	—	—	5	—	5
35–40	37	27	14	5	3	—	—	5	—	—
45	65	34	14	8	3	2	3	5	6	3
50–55	39‡	36	13	10	5	—	—	—	5	—
60–75	12	25	42	8	8	—	8	—	8	—
Total	175	30	15	7	4	1	2	3	4	2

*Nos. of patients at some dose levels are greater than those in table 1 because some patients were treated at > 1 dose level.
†Patients with acute leukemia were excluded.
‡2 patients with early death were included in analysis of acute toxicity.

and alopecia. These were observed at all doses administered but the frequency appeared to be dose-dependent. The gastrointestinal toxicity was acute, generally mild, and short-lived. In some patients who were followed for a sufficient length of time, the alopecia was reversible. Phlebitis with local pain occurred within 24 hours after drug administration. There was no local burning sensation at the time of administration. Several patients who experienced drug extravasation at the site of administration did not complain of burning pain and there was no tissue necrosis. In these instances, there was associated local swelling with mild overlying cutaneous erythema which promptly responded to application of ice packs. Phlebitis could not be prevented by administering the drug in a large volume of fluid or by post-treatment with more fluid or heparin.

Skin rash or urticaria was uncommon and regularly responded to antihistamines; recurrence was prevented by premedication with antihistamines. Of the seven episodes of fever that developed, three were a result of pneumonia and the remaining four had no identified source. Granulocytopenia was present during all these episodes. One episode was fatal with concomitant hemorrhage secondary to drug-induced thrombocytopenia. There were no deaths related to thrombocytopenia alone. Two patients noticed a peculiar skin odor of peptichemio after receiving each course of treatment which persisted for several days.

Table 3 lists the hematologic toxic effects observed at various dose levels. Only patients who received two or more consecutive courses repeated at the same dose level are included in this analysis. The aim was to detect cumulative bone marrow toxicity, if present. This analysis excludes all patients with acute leukemia who had abnormal peripheral blood counts. The degree of myelosuppression did not appear to be dose-dependent. Also, the day of occurrence of maximum myelosuppression and the duration of myelosuppression did not appear to be dose-dependent. However, among patients who received repeated courses at 45 and 55 mg/m²/day for 3 days, there was cumulative myelotoxicity as evidenced by lower median values for both granulocytes and platelets during the subsequent courses. There were no major differences observed in the median times at which maximum myelosuppression occurred, but the duration of myelosuppression was more prolonged. These changes were more prominent for the platelets than for the wbcs. During subsequent courses, thrombocytopenia occurred later than granulocytopenia, and recovery was more prolonged. It was necessary to reduce the dose in some patients because of this effect.

There were no abnormalities of renal or hepatic function that could be directly attributed to peptichemio therapy. A few patients had mild renal or hepatic dysfunction at the start of treatment. The hematologic and nonhematologic toxic effects did not appear to be more severe in these patients compared with other patients.

Therapeutic Response

The various malignant diseases treated and their responses are shown in table 4. The majority of the 104 evaluable patients had solid tumors. No patient achieved a CR. Seven patients achieved a PR and four patients obtained lesser responses. Six of these 11 patients had melanoma or genitourinary carcinoma (urethra, renal cell, and bladder). The responder with lymphoma had Hodgkin's disease, and the responder with head and neck carcinoma had lymphoepithelioma. All responses were seen in metastases either to soft tissues, lungs, or lymph nodes. These responses were of 4–36 weeks' duration, with the majority of responses lasting 8–20

TABLE 3.—Hematologic toxic effects

Dose (mg/m²/day × 3 days)		Initial count			Subsequent count		
		Wbcs	Neutrophils	Platelets	Wbcs	Neutrophils	Platelets
25	No. of courses	1	1	1	1	1	1
	Lowest count, cells × 10^3/mm³	2.6	2.1	94	1.6	1.0	140
	Day	—	17	20	—	19	19
	Duration*	—	—	9	—	—	—
35	No. of courses	6	6	6	8	8	8
	Median lowest count, cells × 10^3/mm³ (range)	3.3(1.6–4)	2(0.6–2.4)	126(87–220)	2.7(1.9–3.5)	1.3(0.9–1.8)	131(95–190)
	Day (range)	—	21(7–21)	19(14–33)	—	19(7–24)	23(7–30)
	Duration* (range)	—	14	10(6–14)	—	3	12
45	No. of courses	13	13	13	15	15	15
	Median lowest count, cells × 10^3/mm³ (range)	3.2(2.1–8.4)	2(0.7–6.4)	128(42–245)	2.5(0.7–4.5)	1.1(0.2–6.4)	80(15–243)
	Day (range)	—	16(11–24)	21(11–30)	—	15(7–20)	17(7–29)
	Duration* (range)	—	6	6(3–13)	—	6(2–11)	14(5–47)
55	No. of courses	4	4	4	9	9	11
	Median lowest count, cells × 10^3/mm³ (range)	2.6(2.5–4.4)	1.8(1.6–3.5)	130(40–156)	2.4(1.1–4.4)	1.5(0.5–3.7)	74(27–132)
	Day (range)	—	21(15–22)	16(14–21)	—	18(8–34)	24(9–58)
	Duration* (range)	—	—	11	—	3(3–13)	15(6–38)

* No. of days for recovery of polymorphonuclear neutrophils to > 1000/mm³ and/or platelets to > 100,000/mm³.

TABLE 4.—Response by disease category

Disease	No. of patients treated	No. of patients with—					
		PR	Stable disease: Less than PR	Stable disease: No change	Progression	% response	Duration (wks)
Melanoma	22	2	1	2	17	14	8,12; 16; 16,16
Gastrointestinal	19	—	2	1	16	11	9,12; 18
Genitourinary	13	2	1	4	6	23	36,20; 8; 32,8,4,16
Lung	9	—	—	3	6	—	8,10,12
Thyroid	6	—	—	2	4	—	8,5
Breast	5	1	—	—	4	20	20
Lymphoma	5	1	—	2	2	20	10; 16,4
Sarcoma	5	—	—	—	5	—	—
Head and neck	3	1	—	1	1	33	8; 8
Carcinoma, unknown primary	4	—	—	1	3	—	8
Acute leukemia	13	—	—	—	13	—	—
Total	104	7	4	16	75	11	4–36

weeks. One patient with metastatic urethral carcinoma had a response which lasted for 36 weeks.

All responses were observed at doses ≥ 35 mg/m²/day (table 5). Most responses were attained at 45 mg/m²/day. The median time to response was 6 weeks, with a range of 4–12 weeks. Seventy-nine of the 104 patients had previously received alkylating agents. Among the 11 patients who achieved a response, seven had prior exposure to such agents (cyclophosphamide, mechlorethamine, or the nitrosoureas) within 2–4 months of starting peptichemio. Five of these seven patients achieved a PR.

DISCUSSION

In this study, the dose of peptichemio of 45 mg/m²/day for 3 days (135 mg/m²/course) repeated at 3–4-week intervals appears to be appropriate in terms of tolerance and antitumor activity. The most frequent important side effects were myelosuppression

TABLE 5.—Response and dose

Dose (mg/m^2/day × 3 days)	Disease	Response	Time to response (wks)
35	Gastrointestinal	1 less than PR	6
45	Head and neck	1 PR	4
	Breast	1 PR	12
	Gastrointestinal	1 less than PR	6
	Lymphoma	1 PR	9
	Melanoma	1 PR; 1 less than PR	4; 8
	Genitourinary	2 PRs; 1 less than PR	5,5; 8
55	Melanoma	1 PR	4

(polymorphonuclear neutrophils $< 1000/mm^3$ and/or platelets $< 100,000/mm^3$, which was unpredictable) and troublesome phlebitis, both of which were observed at all dose levels tested. Approximately 85% of all patients treated with peptichemio developed myelosuppression. This marrow toxicity was cumulative and could be prolonged, necessitating delay of subsequent therapy. This was particularly pertinent with regard to platelet recovery.

Phlebitis could not be prevented with various approaches (a larger volume of iv fluids administered over a longer period of time or post-treatment flush with fluids or heparin). In general, the side effects we encountered are in agreement with those reported by other investigators (8,14).

We did not note striking antitumor activity against any particular malignancy tested, although most of the responses were observed in patients with melanoma and genitourinary carcinoma. All patients treated had far-advanced metastatic disease and had had extensive prior therapy with other chemotherapeutic agents plus other treatment modalities. It is likely that peptichemio will be found to have greater activity if used earlier in the treatment of those malignancies in which responses have been observed. The responses seen were obtained as early as after one course of therapy, although the duration of response usually was relatively short. It would appear logical to give this drug to patients at an earlier stage of their disease when the tumor burden is lower. Peptichemio has been administered to patients with solid tumors in combination with other known active agents and the results are encouraging.[4] Further studies are in progress to define more clearly the activity of peptichemio in patients with less far-advanced disease, to compare its activity with that of melphalan, and to ascertain its activity in combination with other antitumor agents.

[4] Robustelli Della Cuna G, Bernardo G, Strada M, et al. Clinical trials with peptichemio (PTC) in the palliative treatment of solid tumors in advanced stage. Unpublished data from Fondazione Clinica de Lavoro, University of Pavia, Italy.

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cis-Dichlorodiammineplatinum(II) and Hexamethylmelamine in the Treatment of Non-Oat Cell Lung Cancer: A Pilot Study of the Southeastern Cancer Study Group [1,2]

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SUMMARY

Fifty-one patients with metastatic non-oat cell carcinoma of the lung were treated with a combination of *cis*-dichlorodiammineplatinum(II) and hexamethylmelamine. The overall response rate (all cell types) was 16%. Four of 20 patients with adenocarcinoma had a partial response and an additional five patients classified as having stable disease had tumor regression < 50%. The median survival of responders and of those with stable disease (all types) was 8 and 7 months respectively, significantly longer than the median survival of patients who progressed (median survival, 2 months [$P < 0.05$]). The major dose-limiting toxicity was nausea and vomiting in over half of the patients; hematologic toxicity and peripheral neuropathy were the other adverse effects of the combination.

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The chemotherapy for metastatic non-oat cell lung cancers is characterized by low response rates (8%–25%) and short median survival times for responders (6–9 months) (1,2). As part of our effort to develop more effective chemotherapeutic regimens for these diseases, we studied a combination including hexamethylmelamine (HMM) and *cis*-dichlorodiammineplatinum(II) (CDDP). When used alone, HMM has produced an average response rate (complete plus partial [PR] response) of 18% in non-oat cell lung cancer (3). As a single agent, CDDP produced a PR in two of 39 patients with lung cancer in one series (4), while other series have reported occasional responses (5). Our pilot study of HMM and CDDP was initiated to determine the feasibility of combining these two agents and to test the efficacy of the combination in non-oat cell lung cancers.

METHODS

Patient Selection

Patients with recurrent and/or metastatic adenocarcinoma, squamous cell carcinoma, or large cell undifferentiated carcinoma of the lung were entered in the study if they had at least one measurable lesion, if their performance status (PS) (Karnofsky scale) was ≥ 40%, and if their anticipated survival was ≥ 6 weeks. Diagnosis was by histology in 47 patients and by cytology alone in four. Evaluation for staging included, in addition to physical examination and chest roentgenograms, liver function tests, liver and bone scans, and, where indicated, chest tomograms, brain scan, and bone marrow biopsy. Thirty patients had extrathoracic metastases, while 21 had pulmonary and/or intrathoracic metastases. A total of 36 males, ranging in age from 45 to 71 years (median, 56), and 15 females, ranging in age from 43 to 75 years (median, 57), were studied.

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Protocol Design

Initially, patients received CDDP at a dose of 50 mg/m^2 by rapid iv infusion every 3 weeks as outpatients. HMM was given orally at a dose of 100 mg/m^2/day in four divided doses (after meals and at bedtime). For the last ten patients entered in the study, the dose of CDDP was increased to 75 mg/m^2 and was given as a 6-hour infusion together with iv hydration, furosemide, and mannitol according to the technique of Higby et al (6). The HMM dose was increased to 150 mg/m^2.

Criteria for response were those of the Southeastern Cancer Study Group: *PR*—regression of ≥ 50% in the product of diameters of measurable lesions; *stable disease*—regression of < 50%, no change, or an increase of < 25% in any pre-existing lesions; and *progressive disease*—appearance of any new lesion or an increase of ≥ 25% in the size of pre-existing lesions.

Toxicity was graded according to Southeastern Cancer Study Group criteria (7). A therapy trial consisted of 6 weeks of treatment: CDDP given on Days 1 and 22 and HMM given daily from Day 1 to 42. All patients were evaluated after completion of the 6-week treatment period. Those patients who showed a response or whose disease proved to be stable received additional cycles of treatment, providing that toxicity did not necessitate termination of therapy.

Informed consent was obtained from all patients prior to entry in the study as required by the Human Use Committees of the participating institutions.

RESULTS

Response to treatment is summarized in table 1 and the median survival from the start of treatment is summarized in table 2. The overall response rate for all cell types was 16%. No complete responses were observed in any disease category. There were four PRs in patients with adenocarcinoma; five of eight patients listed as having stable disease showed tumor regression < 50%. Thus, nine patients (45%) with this tumor showed some degree of objective response. The median survival of responders was 8.5 months and the median survival of stable-disease patients was 11 months; this difference is not statistically significant. In contrast, patients with progressive disease had a median survival of 1.5 months ($P < 0.05$).

Only one of 19 patients with squamous cell carcinoma had a PR. The median survival of 11 patients with stable disease was four times longer (6.5 versus 1.5 months, $P < 0.05$) than the median survival of patients whose disease progressed.

Three of 12 patients with large cell undifferentiated carcinoma had a PR; in four patients, the disease remained stable. Survival of responders was significantly longer ($P < 0.05$) than survival of patients with progressive disease.

PS at the start of treatment did not influence response except in patients with large cell undifferentiated carcinoma, where the median PS at the start for those who progressed was only 50% compared to a median PS for all patients of 70% (PS range, 40%–100%).

Among the eight responders (all cell types) the sites of response included: lung metastases in six patients, peripheral lymph nodes in two patients, liver metastases in one patient, and subcutaneous metastases in one patient.

A majority of the patients (56%) experienced gastrointestinal toxicity in the form of nausea and vomiting, which was severe enough to require discontinuation of chemotherapy in four patients.

Hematologic toxicity, occurring in 25 patients (49%), consisted of anemia in 14 patients, with a fall in the hemoglobin level of 3–5 g/100 ml in nine patients and > 5 g/100 ml in five others. Eight of nine patients with thrombocytopenia had a fall in platelets to between 50,000 and 100,000/mm^3; only one had a platelet count of < 50,000/mm^3. Of seven patients with granulocytopenia, two had decreases in granulocyte counts to levels of 680 and 625/mm^3 respectively. In most patients, hematologic toxicity appeared only after three full cycles (9 weeks) of

TABLE 1.—Response of non-oat cell lung cancer to treatment with CDDP and HMM

Cell type	No. of patients	No. of responses (%)		
		PR	Stable disease	Progressive disease
Adenocarcinoma	20	4 (20)	8 (40)	8 (40)
Squamous cell carcinoma	19	1 (5.3)	11 (57.9)	7 (36.8)
Large cell undifferentiated carcinoma	12	3 (25)	4 (33)	5 (41.7)

TABLE 2.—Median survival (mos) of patients treated with CDDP and HMM according to response

Cell type	Median survival of—		
	Responders	Stable-disease patients	Progressive-disease patients
Adenocarcinoma	8.5	11	2.5
Squamous cell carcinoma	5	6.5	1.5
Large cell undifferentiated carcinoma	11.5	4.5	2.5
All types combined	8	7	2

treatment, and was reversible in all patients. Despite the known nephrotoxicity of CDDP, only mild and reversible renal toxicity (elevation of creatinine to 1.2 mg/100 ml or BUN to 27 mg/100 ml) occurred in six patients.

The most troublesome toxic manifestation was paresthesias, especially in the lower extremities. This symptom occurred in seven patients and was moderately severe in four of these. The peripheral neuropathy occurred after a minimum of four cycles of treatment (12 weeks) in four patients; in three of these, the condition persisted for months after treatment was discontinued. One patient developed hallucinations and depression after 6 weeks of treatment; these symptoms disappeared when HMM was discontinued. No patient experienced ototoxicity.

No increased toxicity was observed from the higher drug doses given to the last ten patients entered, four of whom had a PR.

DISCUSSION

The results of this pilot study demonstrate that the combination of HMM and CDDP results in moderate toxicity. We were unable to increase the dose of HMM beyond 150 mg/m²/day because of nausea and vomiting which became intolerable when added to the nausea and vomiting associated with administration of CDDP. Both HMM and CDDP alone can produce neurologic toxicity; in combination, significant peripheral neuropathy developed in several patients, which resolved slowly in some and not at all in one patient. Significant renal toxicity was not seen, even with the higher dose of CDDP, but it is not possible to assess the effect of iv hydration and diuresis in minimizing the nephrotoxicity of this agent.

The combination of CDDP and HMM in non-oat cell lung cancer exhibits a degree of antitumor activity similar to the activity obtained with other drug combinations (1). While our study was not designed to answer the question of whether the combination is superior to HMM used as a single agent, the overall response rate of 16% is comparable to the response rate obtained with HMM alone (3).

It is noteworthy that the median survival of responders was not significantly different from the median survival of patients with stable disease, although both had a better survival than those patients with progressive disease. Some patients in the stable-disease group did experience some objective tumor response, which may account for the improved survival in this group compared to the survival of patients with progressive disease. Alternatively, the group with stable disease may simply represent a population with less aggressive disease, and hence more prolonged survival, unrelated to the effect of chemotherapy.

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Carcinoma of the Anus Treated With a Combination of Radiotherapy and Chemotherapy [1,2]

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The treatment of squamous cell tumors of the anus with a combination of chemotherapy and radiotherapy has recently been described for both resectable (1,2) and unresectable (3) disease. The chemotherapy consisted of 5-fluorouracil (5-FU) given as an iv infusion and mitomycin C.

We report here three patients with squamous cell carcinoma of the anus who, after failing attempted curative resection, were treated with modifications of these regimens. These are the first reported cases of patients with regional disease after surgery who have received this treatment and have remained in continuous complete remission for > 1 year. The results indicate the ability of these modalities to produce complete remissions in patients failing to respond to surgery, and suggest the potential ability to cure such patients.

CASE REPORTS

Patient 1

A 54-year-old female with a past history of rheumatic heart disease, atrial fibrillation, congestive heart failure, and a right hip plate for tuberculous arthritis presented in November 1974 with rectal pain and bleeding of 6 months' duration. Biopsy of an anorectal mass revealed a cloacogenic carcinoma of the basaloid type, and the patient underwent an abdominoperineal resection.

In August 1975, a posterior vaginal wall recurrence was noted. Attempted local resection was unsuccessful. The patient was referred for further treatment because of tumor growth causing increasing pelvic and perineal pain.

On April 24, 1976, she began receiving combination chemotherapy consisting of mitomycin C (5 mg/m^2) by bolus iv injection on Days 1 and 8 and 5-FU (30 mg/kg/day) via constant iv infusion for 5 days. This course was to be repeated every 5–6 weeks. A conservative low-dose radiotherapy schedule was employed because we had no prior experience in the use of radiotherapy with mitomycin C and 5-FU infusion. Whole-pelvic radiotherapy via a 360° rotational field was started on May 11, 1976. The patient was treated with cobalt 60 using a 12 × 7-cm field at the axis of rotation, delivering a dose of 3000 rads in 15 sessions.

Upon complete examination at the end of the second course of chemotherapy, there was no evaluable disease. The patient remains in complete remission with no demonstrable tumor. Consideration is being given to discontinuing the chemotherapy.

There was no toxicity secondary to the radiotherapy. Side effects attributable to the chemotherapeutic regimen have consisted of one episode of mild stomatitis, leukopenia (2000 cells/mm^3), and thrombocytopenia (70,000 cells/mm^3), necessitating intermittent reduction by 50% of the mitomycin C dose by discontinuing the Day-8 dose. One episode of fever to 104°F may have been due to mitomycin C. There was no evident source for the fever and it resolved without specific therapy. The patient has never been symptomatic due to bone marrow depression, and the interval between courses of chemotherapy has never been prolonged because of the side effects noted.

Patient 2

A 39-year-old female was hospitalized in September 1976 for an anal mass and an enlarged left inguinal node. Biopsy of the anal mass revealed an infiltrating squamous cell carcinoma. Blood chemistry tests, chest roentgenogram, iv pyelogram, barium enema, and liver scan were normal. Because of

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a past history of carcinoma in situ of the cervix treated by conization in August 1974, the patient underwent dilatation and curettage which was normal.

In October 1976 an abdominoperineal resection, a total abdominal hysterectomy, and a left superficial groin dissection were performed. There was extensive residual disease with involvement of the femoral, inguinal, external iliac, perirectal, and perisigmoid nodes. There was, however, no clinically measurable disease after surgery.

Chemotherapy consisting of 5-FU (30 mg/kg/day) via continuous iv infusion for 5 days and mitomycin C (10 mg/m^2) on Day 1 was started in December 1976. This course was repeated every 5–6 weeks. Whole-pelvic radiotherapy, identical to the treatment plan used in patient 1, was administered during the first course of chemotherapy.

After 11 courses of chemotherapy, the patient refused further treatment for social reasons. She presently remains asymptomatic except for minimal swelling of the left leg which has been unchanged since surgery. There is no evidence of recurrent or residual disease. Recent liver and bone scans and abdominal and pelvic sonograms are normal.

There was no pelvic toxicity secondary to the radiation therapy. The principal toxic effect has been reversible thrombocytopenia (22,000 cells/mm^3) after the fourth course of chemotherapy. Because of this, mitomycin C was omitted during the next two courses and then reduced to 7.5 mg/m^2. During the first course of chemotherapy, mild stomatitis and diarrhea necessitated discontinuation of the 5-FU infusion after 3 days. The patient tolerated the full 5-day 5-FU infusion on all subsequent courses. The interval between administration of 5-FU was never prolonged because of toxicity.

Patient 3

A 39-year-old male presented in March 1977 with a right-sided ulcerated anal mass. This was surgically excised and reported elsewhere as condyloma acuminata. The lesion failed to heal, and a sinus tract formed. In April 1977, re-operation with deep excision into muscle did not fully remove the gross tumor. Pathologic tests showed a squamous cell carcinoma. Chest roentgenogram and liver and bone scans were normal.

In June 1977, chemotherapy consisting of 5-FU (30 mg/kg/day) via constant iv infusion for 5 days and mitomycin C (10 mg/m^2) given iv on Day 1 was started. Concomitantly with the chemotherapy, a single-plane radium implant was performed utilizing 4.5-cm active length needles and delivering a dose of 6000 rads in 151 hours to the tumor. Because of palpable inguinal lymphadenopathy, the patient was also given radiotherapy to the groin and iliac nodes by means of right and left 12 × 7-cm fields on a 4-MEV linear accelerator, delivering a dose of 4500 rads in 15 sessions. After the second course of chemotherapy, the patient refused further treatment. In December 1977, a mass appeared at the previous surgical site. The excised mass consisted of granulated tissue with no evidence of residual tumor.

The patient is presently asymptomatic. The anal scar is well-healed and there is no evidence of disease.

There were no significant toxic effects secondary to the radiotherapy. A platelet nadir of 88,000/mm^3 occurred 1 month after the second course of chemotherapy, but there were no other side effects due to chemotherapy.

DISCUSSION

Squamous cell anal neoplasms including the histologic variant cloacogenic carcinoma are relatively uncommon, comprising only 2.7%–12% of all anorectal tumors (4–7). The prognosis of these tumors and their response to different modes of treatment are poorly defined because of both the low incidence of the disease and the different staging systems and histologic classifications used in different reports (6,8,9).

Surgery has generally been considered the best treatment. The 5-year survival rate for patients with resectable tumors has been approximately 45% (7–10). In contrast, the prognosis for patients with unresectable or recurrent disease has been poor with both surgery and radiotherapy. Klotz et al (6), in a review of 373 cases of cloacogenic carcinoma, described a mortality rate of 91%–100% for patients with recurrences treated by radiotherapy and/or chemotherapy. Failes et al (11) reported no 5-year survivors in patients with clinically involved nodes. Kheir et al (12) concluded that patients with perineal or pelvic recurrence died within 2 years regardless of the treatment used. Their review includes two patients with pelvic recurrences and no other evidence of metastatic spread who were treated with 6000 rads of cobalt 60 administered to the pelvis over 6 weeks.

Chemotherapy alone has only occasionally been of palliative benefit. Lindell et al (13) reported partial responses lasting from 4 to 13 weeks in three of six patients treated with either bleomycin alone or bleomycin and CCNU. Five of these patients had no previous radiotherapy. Other reports of chemotherapy experiences include two failures of pelvic infusions with mechlorethamine (14), one partial re-

sponse to a combination of vincristine and bleomycin (15), one failure of hepatic artery 5-FU infusion (12), and three failures of 5-FU bolus regimens (12,16). Mackman et al (17) reported two patients with unresectable local disease who responded to intra-arterial infusion of 5-FU. Two responses have recently been reported by Quan et al (2) with iv 5-FU infusion and mitomycin C used without radiotherapy. We are unaware of any reports of activity of mitomycin C used alone in this disease. One patient who failed to respond to adjuvant bleomycin, 5-FU bolus, and mitomycin C subsequently had an 8-month partial response to adriamycin, followed by a 4-month partial response to *cis*-dichlorodiammine-platinum(II) (18). Chemotherapy failures have often occurred in sites of nonirradiated tumors.

The benefit of external radiotherapy alone in the treatment of squamous cell carcinoma of the anus is limited. The consensus appears to be that this tumor usually requires treatment with 5000–6000 rads when a good response is achieved, but that external irradiation of recurrent or unresectable disease has been palliative at best (11,14,19–21). Excellent results with the use of interstitial irradiation for localized lesions have been reported by Papillon (10) from France and Dalby et al (21) from England. These studies, however, lack a standardized or well-defined staging system. Doses of 5500 or 6000 rads were usually required for local sterilization. Dalby et al (21) concluded that for patients with involved inguinal lymph nodes, the results of external irradiation alone are so poor that this method should never be preferred to surgery in potentially curable cases.

Buroker et al (1) and Quan et al (2) have recently reported the preoperative use of radiotherapy with 5-FU infusion and mitomycin C in patients with resectable anal carcinoma. The majority of the patients received 3000 rads to the pelvis, one dose of mitomycin C, and one or two courses of 5-FU infusion prior to surgery. Buroker et al (1) reported that in all six of their patients with neoplasms not >4 cm in diameter no tumor was found in the operative specimens.

Newman et al (3) reported on the use of similar regimens in four patients with inoperable squamous cell carcinoma of the anus. One patient died of *Candida* sepsis during radiotherapy. A second patient had a complete response and was treated with monthly courses of mitomycin C and 5-FU. A metastatic tumor appeared in the scrotum after 10 months. The third patient was deemed operable after radiotherapy and one course of chemotherapy. Subsequent abdominoperineal resection revealed no residual carcinoma. At last report, this patient has survived $3\frac{1}{2}$ years with no evidence of disease. The fourth patient had pulmonary metastases which responded to 5-FU infusion and mitomycin C alone. The reported followup was only 6 weeks.

The patients we report in this communication are the only ones we are aware of who, after failing attempted surgical resection because of regional disease, have been treated with this regimen and have remained in complete remission. Of possible significance is that the dose of 5-FU used in our patients is higher than that used by the other investigators of this regimen (1–3).

The doses and technique of the radiotherapy given to patient 3 were selected for two reasons. The absence of any radiation toxicity in patients 1 and 2 suggested we could increase the dose from the low-dose schedule employed in the first two patients. The primary tumor in patient 3 was amenable to the use of interstitial irradiation. Based on our experience and that cited in the literature (10,21), we felt this to be the preferred method of treatment for the primary tumor. The results in all three patients demonstrate the ability to use simultaneous multimodality regimens with very acceptable toxic effects.

At this time, the contributions of each of the modalities or treatment schedules to the total effectiveness of this regimen remain undefined. Some additive or synergistic effect is highly likely. In the dosages used, radiotherapy alone is very unlikely to have produced the results observed in these patients. Similarly, 5-FU infusion, with or without mitomycin C, is unlikely to have produced these sustained complete remissions.

To date, these tumors have been responsive to 5-FU when given by infusion but not when given by bolus. 5-FU has a different pattern of side effects depending on its mode of administration (22). As a bolus, it produces dose-limiting myelotoxicity; as an infusion, it produces dose-limiting gastrointestinal and epithelial toxicity. 5-FU given as an infusion, either alone or in combination, may deserve testing against a variety of epithelial tumors where 5-FU bolus regimens have only been marginally effective. Similarly, this combined modality approach deserves consideration for testing against several regional tumors which are *partially* responsive to both the radiotherapy and chemotherapy. Such trials are in progress for recurrent carcinoma of the rectum but are not yet in progress for carcinoma of the bladder or oropharynx.

Squamous cell carcinoma of the anus has been a disease in which treatment of operable patients has been a source of considerable morbidity and treatment of inoperable patients has usually been futile. Although it is too early to define the long-term benefit of these regimens, results in these three

patients with unresectable tumors are clearly better than past experience with single modalities. Thus, this multimodality regimen deserves further development and testing.

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Hypersensitivity Reactions to Iv Melphalan During Treatment of Multiple Myeloma: Cancer and Leukemia Group B Experience [1,2]

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SUMMARY

Ten patients developed allergic reactions to iv melphalan (L-PAM) during therapy for multiple myeloma. The incidence of such reactions was 2.4% among 425 patients receiving iv L-PAM with or without other drugs and 3.9% among 255 patients receiving iv L-PAM alone. Only one such reaction was demonstrated in 294 patients who initially received oral L-PAM. The median day of first reaction to iv L-PAM was Day 222 (range, Days 44–909) and the median total dose prior to a reaction was 185 mg (range, 51–520 mg). Of five patients who subsequently received oral L-PAM, four developed a reaction similar to that experienced with the iv drug.

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Melphalan (L-PAM) has long been used as a first-line drug in the treatment of multiple myeloma. In several large series reporting the use of oral L-PAM, no allergic reactions were reported except for the rare occurrence of a skin rash (1–4). Between 1971 and 1977, iv L-PAM was used in three completed randomized studies of multiple myeloma sponsored by Cancer and Leukemia Group B (CALGB) (protocol Nos. 7161, 7261, and 7461). Informed consent was obtained prior to patient entry in each study. Figure 1 shows the treatment programs for each of the three studies containing iv L-PAM. L-PAM was initially dissolved in 10% ethanol and diluted to a concentration of 10 mg/ml in normal saline and injected slowly (1–2 minutes) directly iv or into the tubing of a running iv infusion. In all three trials, one group of patients was randomized to receive oral L-PAM (0.15 mg/kg/day for 7 days, with a maintenance dose of 0.05 mg/kg/day after recovery from the nadir of the peripheral wbc count) together with an initial 6–10-week course of oral prednisone in tapering doses.

Adjustment of all L-PAM doses was made on the basis of peripheral wbc or platelet counts and, in study 7461, on the basis of elevated BUN or creatinine levels. Half of the patients in study 7461 were

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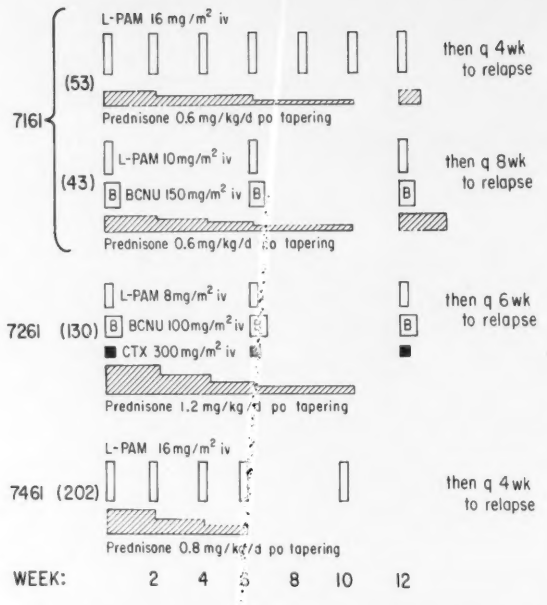
L-PAM 16 mg/m² iv
then q 4 wk to relapse
(53)
7161
Prednisone 0.6 mg/kg/d po tapering
L-PAM 10 mg/m² iv
then q 8 wk to relapse
(43)
B BCNU 150 mg/m² iv B
Prednisone 0.6 mg/kg/d po tapering
L-PAM 8 mg/m² iv
then q 6 wk to relapse
7261 (130)
B BCNU 100 mg/m² iv B
CTX 300 mg/m² iv
Prednisone 1.2 mg/kg/d po tapering
L-PAM 16 mg/m² iv
7461 (202)
then q 4 wk to relapse
Prednisone 0.8 mg/kg/d po tapering
WEEK: 2 4 6 8 10 12

FIGURE 1.—Designs for treatments using iv L-PAM in 3 CALGB myeloma studies (7161, 7261, and 7461). Nos. in parentheses = Nos. of patients.

randomized on Day 154 to receive vincristine (1 mg/m²) and prednisone (0.6 mg/kg for 7 days) every 8 weeks in addition to previous therapy.

RESULTS

During the course of study 7161, one of 96 patients receiving iv L-PAM developed a hypersensitivity reaction (patient 1, table 1). This patient was receiving iv L-PAM alone. In study 7461, nine of 202 patients receiving iv L-PAM developed similar reactions. The incidence of allergic reactions in patients receiving iv L-PAM alone was not significantly different for the two studies (2% versus 4%). Two patients in study 7461 were also receiving intermittent doses of vincristine, but neither received this drug within 1 month of the time the reaction occurred. The serum myeloma immunoglobulin in four of eight patients was the IgAκ type. No adverse effects were reported in 130 patients in study 7261 who received lower-dose iv L-PAM in combination with BCNU and cyclophosphamide. One of the 294 patients who received oral L-PAM alone in these three studies had an allergic reaction. This patient, who had IgAκ-type myeloma, developed generalized edema, urticaria, and exanthema within several days of starting maintenance therapy (3.5 mg/day orally). The drug was then permanently discontinued and the allergic manifestations promptly resolved.

CASE REPORTS

Patient 1

Patient 1 suddenly felt ill on Day 223 while receiving 10 mg of iv L-PAM. The patient was placed in a supine position and became profusely diaphoretic without pulse. A transient tremor of the jaw was noted. A slow heart rate was maintained without arrhythmia and symptoms abated spontaneously within 5 minutes. The patient was subsequently switched to oral L-PAM (3 mg/day) and no further adverse reactions were experienced.

Patient 2

Patient 2 developed severe chest pain, dyspnea, generalized tingling, itching, and nausea while receiving 6.4 mg of iv L-PAM on Day 455. T-wave changes on ECG were noted. On Day 483, an infusion of 6.4 mg of iv L-PAM in 250 ml of normal saline was started, and a similar severe reaction

TABLE 1.—Hypersensitivity reactions to iv L-PAM (CALGB studies 7161 and 7461)

Patient No.	Age/sex	Serum myeloma protein	Date treatment started	Day of reaction	Doses before reaction		Subsequent reaction to oral L-PAM
					No.	Total (mg)	
1	52/F	IgAκ	4/72	223	10	293	No
2	67/F	Free λ	3/75	455	17	261	Yes
3	62/F	IgGλ	6/75	132	7	105	—
4	68/M	IgGλ	7/75	335	13	520	—
5	54/F	IgAκ	7/75	843	28	485	—
6	61/M	IgAκ	7/75	44	2	51	Yes
7	49/F	IgAκ	9/75	140	6	152	Yes
8	65/M	IgGλ	11/75	909	23	589	—
9	60/M	IgGκ	3/77	221	9	185	Yes
10	51/M	None	7/77	222	8	173	—

occurred after 30 ml was infused. The iv infusion was stopped, and treatment with oral L-PAM was started 3 weeks later. Two hours following a single 2-mg dose, she again developed chest pain and the L-PAM was discontinued permanently. She was subsequently changed to cyclophosphamide and prednisone and has had no further allergic reactions.

Patient 3

Patient 3 received a 14-mg dose of iv L-PAM on Day 132 and immediately thereafter had swelling of the fingers with generalized urticaria and pruritus. This reaction abated spontaneously and the patient was not given any further therapy with this drug.

Patient 4

Patient 4 received 40 mg of L-PAM iv on Day 335 and immediately complained of a "funny feeling" across his chest with shortness of breath and a sense that he was going to die. He was given oxygen therapy for 30 minutes and recovered without any further form of therapy. No further L-PAM was given thereafter.

Patient 5

Patient 5 developed an erythematous, pruritic rash with urticaria over his entire body 1–5 minutes after receiving 17 mg of iv L-PAM on Day 329. The reaction cleared completely following two injections of epinephrine (0.3 ml, 1:1000 sc), hydrocortisone (200 mg iv), and diphenhydramine (50 mg orally). Despite additional iv L-PAM no further reactions occurred until Day 843 (dose 29) when he developed a vasovagal attack with a fall in blood pressure and sweating immediately after receiving 17 mg of iv L-PAM. He received diphenhydramine (50 mg iv), after which he had difficulty in breathing, chest pain, and persistently low blood pressure (80/50 mm Hg). He gradually improved over a 3-hour period and was not given any further L-PAM.

Patient 6

Patient 6 had an initial adverse reaction on Day 44, the morning after he received a 34-mg iv dose of L-PAM. At that time, he experienced a "prickly" sensation in his skin, involving the trunk, arms, and face. On Day 71, about 1 hour after another 34-mg iv dose of L-PAM, he had flushing of the thorax, arms, and face, severe discomfort from a subcutaneous "sticking" sensation, and 3 days of facial, thoracic, abdominal, and arm edema. The skin symptoms persisted for 1 week and were aggravated by exposure to sunlight. Following 100 mg of oral diphenhydramine on Day 91, he developed flushing and edema but no skin discomfort after receiving an additional iv dose of 34 mg. On Day 127, he began receiving oral L-PAM. During the first 2 weeks, he had a mild prickly sensation in the skin but had no further difficulty while receiving diphenhydramine.

Patient 7

Patient 7 developed itching of her palms and soles for 1 hour within several minutes after injection of iv L-PAM (20 mg) on Day 140. Mild itching of the palms recurred shortly after an iv injection of 12 mg on Day 168 and of the palms and soles after 20 mg on Day 194. On Day 223, she had an immediate reaction after a 16-mg dose, consisting of palmar and plantar itching, perioral paresthesias, swelling of the tongue and eyelids, and an erythematous rash on her trunk and extremities. She then developed respiratory difficulty without wheezing, and her blood pressure fell to 86/50 mm Hg. She was given diphenhydramine (50 mg im) and hydrocortisone (40 mg iv) and recovered after a few minutes. The protocol was terminated, and she began receiving oral L-PAM and prednisone 1 month later. Thirty minutes after taking 4 mg of oral L-PAM, she again developed itching and a tingling sensation in the palms and soles of her feet which persisted for 30 minutes. The L-PAM was then permanently discontinued and the patient began receiving cyclophosphamide.

Patient 8

Patient 8 became nauseated, vomited, lost consciousness, and developed cardiopulmonary arrest while receiving 16 mg of iv L-PAM on Day 909. He was resuscitated successfully, but had subsequent shortness of breath, upper eye deviation, and generalized clonic movement. An ECG taken immediately after the episode showed atrial fibrillation with a ventricular rate of 80–110. The patient received a repeat dose of L-PAM (16 mg iv) on Day 944 and again developed immediate nausea and vomiting, with substernal chest pain, loss of consciousness, and incontinence of urine and stool. Resuscitation was again successful, and the patient was switched to iv cyclophosphamide without further difficulty.

Patient 9

Patient 9 developed a tingling sensation in his extremities, flushing of his chest and face, a blotchy appearance of his skin, and hypotension (60/40 mm Hg) on Day 221 within several minutes following 20 mg of iv L-PAM. He was given diphenhydramine with prompt resolution of the flushing and a gradual improvement of the blood pressure. He had no respiratory difficulty. The protocol was discontinued, and he was subsequently treated with intermittent oral L-PAM and prednisone. During each 7-day course given every 6 weeks, he developed a mildly pruritic maculopapular rash of the abdomen. This rash generally began on Day 3 of therapy and disappeared by Day 11.

Patient 10

Patient 10 complained of dizziness and sweatiness immediately after receiving a dose of iv L-PAM (30 mg) on Day 222. This reaction resolved spontaneously when the patient lay down. On Day 266, he complained of a tingling sensation in his tongue and suddenly had a cardiopulmonary arrest 1–2 minutes after an iv dose of 15 mg. The patient was successfully resuscitated and has not received any further L-PAM.

DISCUSSION

Hypersensitivity reactions are reported in ten of 425 (2.4%) patients with multiple myeloma treated in three clinical trials with iv L-PAM. Such reactions ranged from edema and generalized rash to a full-blown anaphylactic reaction consisting of respiratory insufficiency and hypotension. At the time of the reactions, all patients were receiving only iv L-PAM and had normal renal function.

The initial reaction occurred between Day 44 and Day 909 (median, Day 222) of the clinical trials, following a median previous total dose of 185 mg (range, 51–520 mg). The reactions occurred over a span of 5 years between 1972 and 1977, although seven of ten reactions occurred between March 1975 and November 1975.

Since some patients in study 7461 were also receiving iv vincristine, an analysis was done of the possible contribution of vincristine to the adverse effects of L-PAM. Only two patients (Nos. 2 and 5) received vincristine (1 mg/m^2 every 2 months), and in both patients the vincristine dose was given 1 month prior to the L-PAM reaction.

Death did not occur in any patient as a result of the drug reactions described. In five patients, the drug was stopped permanently. In five patients, treatment was changed to oral L-PAM. In four of these patients, symptoms recurred which simulated the initial adverse reactions. In the other patient, no further difficulty was encountered.

Oral L-PAM has been used widely in the treatment of neoplasms, most commonly myeloma and ovarian and breast carcinomas. Iv L-PAM has been introduced more recently and is still considered an investigational agent. Its continued use in myeloma has been stimulated by evidence derived from CALGB study 7161 that patients receiving iv L-PAM had a longer median survival (36 months) than those receiving oral L-PAM (16 months) (5). Subsequent comparisons in CALGB study 7461 appear to confirm these findings, but definitive conclusions are not yet possible. In addition, recent data have indicated that the absorption of L-PAM when administered orally varies widely from patient to patient (6).

The drug is manufactured by Burroughs Wellcome Co, Research Triangle Park, NC, and 100 mg of powder is dissolved in 1 ml of 10% ethanol containing K_2HPO_4, propylene glycol, and HCl (final pH = 7) prior to dilution in normal saline to a final concentration of 10 mg/ml. A review of the records at the Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md, has not revealed any prior report of L-PAM hypersensitivity.[5] Although initial adverse reactions were considered possibly related to variations in manufacturing specifications or to the diluent provided with the drug, the repeated occurrence of the allergic symptoms in four of five patients receiving subsequent oral L-PAM appears to militate against these possibilities. Although the reaction almost always occurred during or immediately after iv infusion, in one patient (No. 6) it initially appeared the following day. Therefore, in view of the unpredictable nature of the reactions described in these patients, patients receiving iv L-PAM should be monitored closely and warned of possible adverse effects during the 24-hour period following drug administration. In the event of an untoward reaction, however mild, it is recommended that the drug be discontinued and substituted by cyclophosphamide or a nitrosourea.

The mechanism responsible for the hypersensitivity reactions in these patients is unknown. It has been shown previously that an alkylation reaction between L-PAM and serum proteins may occur in vivo (7) and that such altered proteins may serve as new antigens capable of stimulating antibodies to the hapten-protein complex (8). Antibodies to L-

[5]Turrisi AT, III. Personal communication.

PAM-tumor protein complexes have been shown to cause tumor regression in certain animals, provided the tumor has been pretreated with L-PAM (9). It is thus conceivable that antibodies to L-PAM-cellular protein complexes could play a role in the damage to myeloma or normal cells and that the release of such complexes from such damaged cells could initiate a hypersensitivity reaction under certain circumstances.

It is noteworthy that the serum monoclonal immunoglobulin in five of nine patients (55%) demonstrating allergic reactions to L-PAM was IgAκ. This finding is in marked contrast to the fact that only 13% of the total patients with monoclonal immunoglobulins in these studies had the IgAκ type. In view of the possibility that IgA-type immunoglobulins may be particularly susceptible to alkylation by L-PAM, efforts are being undertaken to test this hypothesis.

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Age-Related Pharmacokinetics of High-Dose Methotrexate in Patients With Osteosarcoma [1,2]

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SUMMARY

Plasma methotrexate (MTX) concentrations were determined in 52 patients after 409 infusions of high-dose (HD) (50–250 mg/kg) MTX with citrovorum factor (CF) rescue. In addition, detailed pharmacokinetic studies were conducted in nine of these patients. Plasma drug levels were compared at 6, 24, 48, and 72 hours from the start of MTX infusions in four different age groups (≤ 10, 11–14, 15–17, and ≥ 18 years of age). At the same drug dose, the younger patients had lower plasma MTX levels than the older patients at 6 and 24 hours. This difference increased in significance with increasing MTX dose. However, MTX plasma levels became similar at 48 and 72 hours, regardless of age. The half-life for the first phase of biphasic MTX clearance in children was shorter than that in adults. In addition, the urinary excretion of MTX was faster in younger children at all doses. The younger patients had a greater apparent volume of distribution of MTX after HD infusion. These results indicate that the age-dependent pharmacokinetics can be attributed to greater distribution and elimination of MTX in the younger patients. The potential difference in the metabolism of MTX between children and adults is currently under investigation. Our observations suggest that age exerts a dominant effect on the pharmacokinetics of HD-MTX.

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Age has been considered to be a significant factor when determining the effect of drugs (1,2). However, information concerning absorption, metabolism, and disposition of antineoplastic drugs is generally lacking for children. Some drugs, such as 6-mercaptopurine, are more efficiently handled by children than by adults (3). Limited data suggest that children eliminate methotrexate (MTX) more effectively than most adults (4). In this paper, additional observations on the effect of age on the pharmacokinetics of high-dose (HD)-MTX are reported. Preliminary results have been presented (5); these results also suggested that the type of neoplasm (ie, osteosarcoma versus Burkitt's lymphoma) may also influence HD-MTX pharmacokinetics. Therefore, the present pharmacokinetic studies of HD-MTX were conducted in a single disease entity, osteosarcoma.

MATERIALS AND METHODS

Patients and Treatment

Data were obtained from 330 infusions of HD-MTX given to 39 children with osteosarcoma. These patients were being treated with massive-dose MTX and citrovorum factor (CF) either as part of a prophylactic adjuvant-therapy regimen (COMPADRI-III and -IV) or for metastatic disease. These

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patients ranged in age from 5 to 17 years, with a median age of 13. The doses of MTX were 50, 75, 100, 150, 200, and 250 mg/kg. Fifteen adult patients (18–70 years of age; median age, 24 years) with metastatic osteosarcoma were also studied. These patients received MTX at doses of between 50 and 250 mg/kg for a total of 79 infusions. A physical examination was performed in all of the patients prior to infusions to detect possible infection or presence of mucosal lesions. Sequential multianalyzer (SMA) screening battery results had to be within normal limits (BUN < 20 mg/100 ml, creatinine < 1.4 mg/100 ml, bilirubin < 1.0 mg/100 ml, and SGOT < 50 units). A platelet count $> 150{,}000/mm^3$ and a wbc count $> 2000/mm^3$ with an absolute granulocyte count $> 1500/mm^3$ were required. Creatinine clearance had to be ≥ 90 ml/minute/1.73 m^2. Postinfusion evaluations included mucosal and skin examinations with SMA screening tests and complete hematologic profiles. Intensive supportive therapy was provided when necessary. Informed consent was obtained from the individual and/or parents for both therapy and the sampling of body fluids. MTX was obtained from the Division of Cancer Treatment, National Cancer Institute, Bethesda, Md, packaged as the sodium salt in vials containing either 500 or 1000 mg with no preservatives.

Alkalinization with acetazolamide was started 12 hours prior to infusion concomitant with a single dose of vincristine (2 mg/m^2). MTX at doses of 50–350 mg/kg was infused during 6 hours followed by iv fluids for 48 hours with sodium bicarbonate at doses of 50 mEq/liter. CF was started 3 hours after completion of infusion at a dose of 15 mg iv every 3 hours for a total of nine doses, and then im every 6 hours for the next 48 hours. Urinary pH was monitored closely and was kept within a range of 7.0–7.5 for 72 hours after infusion. Both children and adults were treated using the same clinical protocol. The scheduling of HD-MTX allowed sufficient time for recovery from previous courses of chemotherapy. The concomitant use of other medications that could be hepatotoxic or nephrotoxic was avoided.

Blood and Urine Samples

Venous blood (3 ml) was drawn into an ethylenediamine tetraacetic acid-vacutainer tube before and after the MTX infusion at designated times. Urine specimens were obtained before MTX infusions, during infusions (0–6 hours), and after infusions (6–24 and 24–48 hours).

Determination of Methotrexate and Analysis of Pharmacokinetics

MTX was determined by kinetic enzymic assay and on many occasions was confirmed by radioimmunoassay (RIA) as described previously (6,7). The results of the enzyme assay were reproducible with a 9% deviation within a day and a 13% deviation day-to-day. This method quantitates MTX metabolites and the contaminant to a minor degree (8). RIA was found to have a 0.1% crossover with CF and 7-hydroxy-MTX and a 0.5% crossover with 5-methyl-tetrahydrofolic acid. Higher MTX values were obtained by RIA with a slope of 1.19 against kinetic enzymic assay. In part, this is due to the cross-reactivity with MTX contaminants or metabolites, particularly 4-amino-4-deoxy-pteroic acid (APA). APA and MTX have equal reactivity to the RIA. This has also been observed by Donehower et al (9). 7-Hydroxy-MTX was present in both plasma and urine samples obtained from 6 to 72 hours. APA is a known contaminant of clinical-grade MTX (10). It can be detected occasionally in the patient's plasma and has been isolated from patient urine samples (11). The structure of both 7-hydroxy-MTX and APA has been identified by thin-layer chromatography, high-pressure liquid chromatography, and mass spectrometry. The details of these observations will be published elsewhere. The enzyme dihydrofolate reductase (isolated from *Lactobacillus casei*) used in the tests was obtained from New England Enzyme Center, Tufts Medical School, Boston, Mass. The RIA kit was purchased from Diagnostic Biochemistry, San Diego, Calif.

Postinfusion pharmacokinetics were calculated according to Gibaldi (12). Estimations were based upon a two-compartment open model from postinfusion MTX plasma concentrations.

RESULTS

Analysis indicated that at comparable doses of MTX, adults had higher plasma MTX levels at the end of the 6-hour infusion. Figure 1 is a scattergram of plasma MTX levels in patients receiving the drug at a dose of 150 mg/kg. The older patients (≥ 18 years of age) had higher blood MTX concentrations at the end of the infusion and 24 hours after the initiation of the infusion. The younger patients (≤ 10 years of age) had significantly lower drug levels during the same time period. The MTX levels in patients between 15 and 17 years of age were intermediate between the younger- and older-age groups. However, all levels were superimposable at 48 or 72 hours regardless of patient age.

Studies were conducted during infusion of MTX at doses of 75–250 mg/kg in an effort to further elucidate the relationship between the pharmacokinetics of HD-MTX and the patient's age. MTX plasma levels at 6 and 24 hours were proportionally increased in each age group with increased amount of MTX. At similar MTX doses, the plasma MTX levels at 6 and 24 hours were higher in the older-age groups. However, no significant deviations of MTX levels could be found at 48 or 72 hours with different doses in all age groups. The results obtained from 6- and 48-hour samples are summarized in table 1.

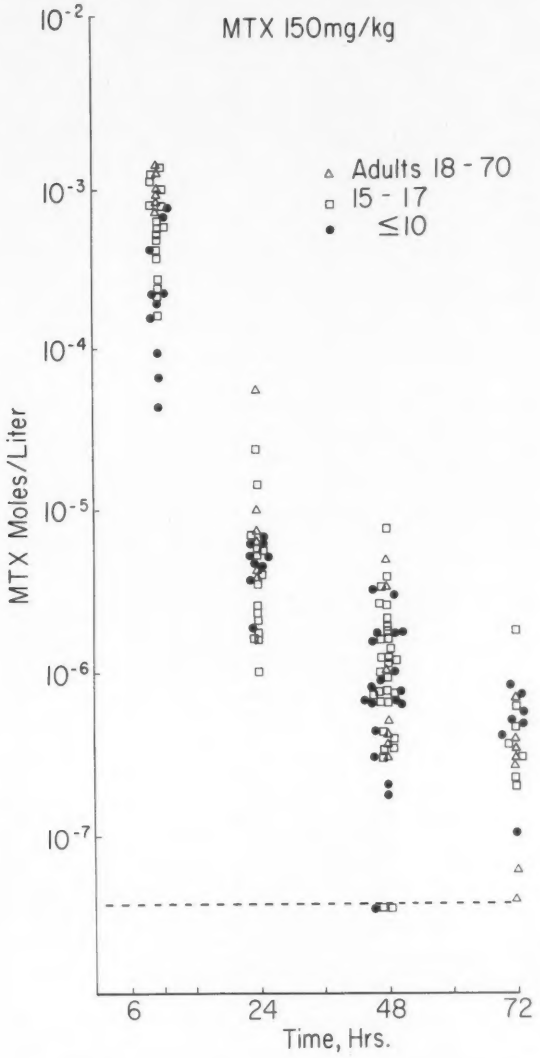

FIGURE 1.—Scattergram of plasma drug concentrations in patients after HD-MTX (150 mg/kg) infusion.

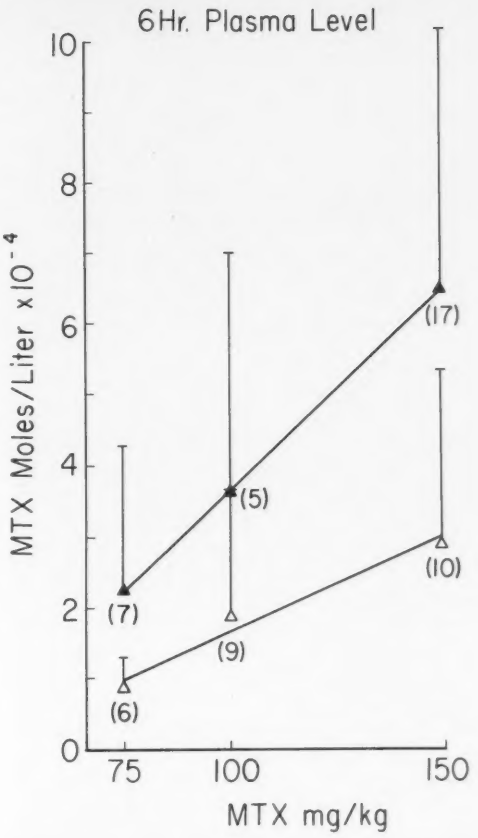

FIGURE 2.—Drug dose and plasma MTX concentration at 6 hrs after start of MTX infusion in patients ≤ 10 yrs of age (△) and in patients 15–17 yrs of age (▲). Nos. in parentheses = No. of observations.

Figure 2 compares the 6-hour plasma MTX level in two age groups: patients ≤ 10 years of age and patients 15–17 years of age. At the dose of 150 mg/kg, the difference in the 6-hour level between the two age groups was highly significant ($P < 0.01$). At 6 hours an MTX infusion of 75 mg/kg given to the older group of patients could achieve a plasma MTX level approximately equivalent to the level achieved by an infusion of 150 mg/kg of MTX given to the younger group. Furthermore, the plasma MTX levels in patients 11–14 years old were intermediate between those of the previous two groups. However, there was no statistically significant difference between plasma MTX levels in the 11–14-year-old group compared to those in either the younger or older groups at any sampling time. The differences

TABLE 1.—Effect of age on plasma MTX levels after HD-MTX infusion*

Hr/drug concentration	Age (yrs)	Dose (mg/kg)					
		50	75	100	150	200	250
$6/10^{-4}$ M	< 10	1.2 ± 0.3† (3)‡	0.9 ± 0.3 (6)	1.9 ± 1.7 (9)	2.9 ± 2.5 (10)	—	—
	11–14	1.5 ± 0.8 (4)	2.7 ± 2.3 (8)	1.1 ± 0.8 (7)	7.8 ± 0.9 (3)	7.7 ± 0.8 (5)	—
	15–17	—	2.3 ± 2.2 (7)	3.6 ± 3.3 (5)	6.6 ± 3.8 (17)	9.5 ± 2.2 (5)	14.3 ± 7.7 (6)
	≥ 18	—	6.7 ± 2.7 (8)	—	17.7 ± 17.9 (9)	21.5 ± 17.9 (14)	—
$48/10^{-6}$ M	≤ 10	1.7 ± 1.7 (5)	1.4 ± 1.7 (8)	0.9 ± 1.1 (13)	1.1 ± 0.9 (19)	0.5 ± 0.3 (16)	0.4 ± 0.3 (6)
	11–14	0.7 ± 0.8 (9)	1.3 ± 1.1 (10)	0.6 ± 0.4 (6)	0.7 ± 0.8 (16)	1.4 ± 2.1 (21)	1.2 ± 2.3 (10)
	15–17	—	1.0 ± 0.8 (8)	1.1 ± 1.3 (17)	1.5 ± 1.6 (28)	0.9 ± 0.8 (26)	2.1 ± 2.8 (16)
	≥ 18	—	0.6 ± 0.7 (9)	—	1.5 ± 1.7 (16)	0.6 ± 0.7 (25)	2.6 ± 9.3 (19)

*Figures in the body of the table are molar concentrations, either $\times 10^{-4}$ or $\times 10^{-6}$, as indicated in the column on the left.
†Mean ± 1 SD.
‡Nos. in parentheses = No. of observations.

were significant when plasma levels at 6 and 24 hours were compared with those of adults. There was no significant difference between adults and the 15–17-year-old children. Even with a limited number of infusions in patients 5–70 years of age, a significant correlation between age and plasma drug concentration can be obtained in samples obtained at either 6 or 24 hours from the start of infusions. At a dose of 75 mg/kg, the correlation coefficient is 0.3340 for 29 infusions ($0.10 < P < 0.05$) at 6 hours and 0.4277 for 24 infusions ($P < 0.05$) at 24 hours. With an MTX dose of 150 mg/kg, the correlation coefficient is 0.5358 ($P < 0.001$) at 6 hours for 38 infusions and 0.3702 ($P < 0.05$) at 24 hours for 33 infusions. In this particular study, the age distribution is very even among the four age groups (≤ 10, 11–14, 15–17, and ≥ 18 years of age). Since the dose administered was calculated on the basis of mg/kg of body weight, the ratio of body weight:body surface area (BW:BSA) was examined. With 17 observations in the 15–17-year-old age group infused with 150 mg/kg of MTX, the BW:BSA ratio was about 1.25 times that of the ten observations in the ≤ 10-year-old group infused with a similar dose. This ratio was not in accord with a 2.28-fold increase of plasma MTX level obtained in the older children.

One of the factors contributing to the lower plasma MTX levels for younger children appears to be a better excretion of this drug during the treatment regimen. Furthermore, it is calculated that the apparent volume of distribution of the drug is higher in children than in adults. Table 2 summarizes the postinfusion pharmacokinetic analyses of six adult patients (18–70 years of age; median age, 35 years) compared with three younger patients (14–15 years of age; median age, 14 years) receiving MTX at a dose of 75 mg/kg. The $t^{1}/_{2}\alpha$ was calculated to be 70.0 ± 9.2 minutes for the younger patients and 132.5 ± 44.9 minutes for the older patients; this difference is significant ($P < 0.05$). Among the six adults, two of the younger adults (18 and 21 years of age) had lower $t^{1}/_{2}\alpha$ at 90 and 72 minutes respectively. The calculated elimination rate constant and the transfer rate constant from the central compartment (plasma) to tissue compartment of the drug were higher in the children than in the adults. Furthermore, the apparent volume of distribution of the drug was greater in children than in adults. The urinary creatinine clearance in the six adults was 87.8 ± 51.3 ml/minute/m² compared to 115.5 ± 39.8 ml/minute/m² for the three children; this difference is not significant. Children excrete about 60% of their MTX dose during the period of infusion and most of the remaining 40% over the following 18 hours. On the other hand, adults excrete about 40%

TABLE 2.—Postinfusion pharmacokinetic parameters of MTX in patients receiving HD (75 mg/kg) infusions

	Patients	
	Children	Adults
No. of patients	3	6
Median age (yrs)	14	35
$t^{1}/_{2}\alpha$	1.2 hrs	2.2 hrs
α	0.598 hr^{-1}	0.315 hr^{-1}
$t^{1}/_{2}\beta$	10.4 hrs	8.4 hrs
β	0.067 hr^{-1}	0.083 hr^{-1}
Kel*	0.578 hr^{-1}	0.306 hr^{-1}
K_{12}†	0.018 hr^{-1}	0.007 hr^{-1}
K_{21}‡	0.069 hr^{-1}	0.085 hr^{-1}
$(Vd)\beta$§	1.1 liters/kg	0.5 liter/kg

*Kel = elimination rate constant of the drug.
†K_{12} = transfer rate constant from the central compartment to the tissue compartment.
‡K_{21} = transfer rate constant from the tissue compartment to the central compartment.
§$(Vd)\beta$ = apparent volume of distribution.

of their MTX dose during the period of infusion and most of the remaining 60% over the following 18 hours (table 3).

DISCUSSION

The influence of age on drug pharmacokinetics has been known for some years. Children, except for newborns and infants, seem to be more efficient in drug absorption, metabolism, and excretion (1,2). Freeman-Narrod et al (4) observed two different patterns in the elimination of MTX in man. In their study, some of the adults and all of the children showed a relatively rapid plasma clearance with a half-life of 90 minutes after iv administration of MTX. Under the same conditions, the adults had plasma half-lives of 6–8 hours. Our results suggest that even with alkalinization and hydration, the difference in plasma clearance between children and adults still exists. Although the MTX dose in this study was based on mg/kg of body weight, the adjustment from BW to BSA did not alter the results. Furthermore, regardless of dose, the urinary excretion of MTX in children was faster than in adults. Freeman-Narrod et al (4) correlated differences in urinary creatinine clearance with the rapid or slow elimination of MTX from the plasma. The urinary excretion data and plasma clearance of MTX in our report clearly suggest that children eliminate MTX faster than adults. Although there is no direct evidence to support the difference among various age groups within pediatric populations, younger children appear to eliminate MTX better than older children based on plasma MTX levels at 6 and 24 hours. Other possible contributing factors such as greater tissue distribution or absorption in various organs or increased metabolism of MTX could not be determined. However, from the pharmacokinetic analyses, the apparent volume of distributions is greater in children than in adults at an MTX dose of 75 mg/kg. The possible difference in plasma binding of MTX between children and adults has not been investigated. Other aspects regarding the metabolism of HD-MTX in man have been reported (6,13).

The $t^{1}/_{2}\alpha$ and $t^{1}/_{2}\beta$ obtained in adult patients (median age, 35 years) are similar to those reported by Isacoff et al (14), although the percent of urinary excretion during the first 24 hours is substantially different. In our study, > 90% of the MTX was excreted during the first 24-hour period from the start of MTX infusions regardless of age, as contrasted to the 60% 24-hour excretion reported by Isacoff et al. The main differences between these two treatment regimens are the administration of MTX in 4 hours and the forced hydration used by Isacoff et al (14) which resulted in a fivefold lower plasma MTX level at 24 hours.

The age-related MTX pharmacokinetics are also correlated with clinical MTX toxicity (15). Although the younger children have less frequent episodes of severe toxicity following HD-MTX, the toxicity among them is dose-related. The older children have a higher frequency of significant toxicity but the toxicity is not dose-related.

Patients developing moderate and severe toxicity during HD-MTX-CF therapy tend to have high plasma levels of MTX at 48 hours (11,15). Therefore, the duration of exposure to high concentrations may be significantly implicated in the occurrence of toxicity.

Another factor that appeared to be related to toxicity was the number of prior HD-MTX infusions. The patients who had ten or more prior infusions of HD-MTX were at significantly higher risk of developing moderate to severe toxicity (15). In the present study, the plasma MTX level at the end of infusion (at 6 hours) increased with increasing dose (fig 1), but the 6-hour level was consistently higher

TABLE 3.—Urinary excretion of HD-MTX

Age group (yrs)	Median age (yrs)		% excretion of total drug infused (hrs)		
			0–6	6–24	24–48
≤ 15	10	M*	59.8	41.7	2.7
		SD†	23.3	25.8	1.8
		N‡	17	14	16
≥ 18	24	M*	43.7	64.8	2.3
		SD†	27.3	26.7	1.4
		N‡	10	10	7
		P value	0.10	0.025	0.30

*M = mean of all observations.
†SD = standard deviation.
‡N = No. of observations.

in the older-age group at comparable doses. The mean number of infusions in the older-age group (at a 150-mg/kg dose) was seven compared to the mean number of six prior infusions in the younger-age group. Thus, the difference in the 6-hour plasma level persisted even though the number of prior infusions was similar in the two age categories.

The pharmacokinetic studies were conducted in three children and six adults between October 1976 and February 1977, when both age groups were being given the same doses of MTX. Subsequently, because of increased frequency of toxicity, the adults began receiving MTX at a dose of $\leq$ 150 mg/kg on a 3-week schedule. In the meantime, the MTX doses in children were escalated to $\geq$ 200 mg/kg, most of the doses being given on a weekly schedule. Thus, it is difficult to do additional concurrent comparative pharmacokinetic studies with MTX which require overlapping doses.

The data reported here were obtained both from patients who were apparently free of disease and from patients who were being treated for metastatic disease. The data available at this time do not suggest differences in pharmacokinetics between those patients with and without tumor burdens.

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Chemotherapy for Sarcoma of the Stomach [1,2]

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SUMMARY

The results of chemotherapy for patients with advanced leiomyosarcoma of gastric origin were analyzed. A total of 23 single-agent and multi-drug regimens were given to 17 patients. DTIC and adriamycin (ADR) were the most commonly used chemotherapeutic agents. ADR was used alone or in combination in 13 patients, resulting in partial responses in two patients and disease stabilization in seven. One of the partial responses was obtained with the ADR-DTIC combination and the other was obtained with the cyclophosphamide-vincristine-ADR-DTIC combination. The survival duration for the responders was significantly longer than that obtained for those patients who had disease stabilization or progression. The results of the study show that leiomyosarcoma of gastric origin is less sensitive to chemotherapeutic agents than leiomyosarcomas of other sites, as reported in the literature. New approaches based on the natural history of the disease and with potential to improve the results of treatment of gastrointestinal sarcomas are indicated.

[Cancer Treat Rep 63:411–414, 1979]

Leiomyosarcoma is the most common gastric sarcoma and comprises from 1% to 3% of all gastric cancers. Sarcomas arising from the fibrous, fatty, lymphogenous, endothelial, or neural tissues of the stomach wall are less common (1). Studies of the ultrastructure of leiomyosarcoma indicate that these tumors are composed of poorly differentiated cells with the capacity for differentiation toward smooth muscle (2–5). The overall 5-year survival rate following surgical resection of gastric leiomyosarcoma has been good, ranging from 35% to 54%. However, 5-year survival does not necessarily mean cure because late recurrences do occur.

The current treatment of metastatic leiomyosarcoma of gastrointestinal origin is far from satisfactory. Although objective tumor regressions of $> 50\%$ occur in one half of the patients with leiomyosarcoma of various origin treated with adriamycin (ADR)-DTIC combinations, the response rate for gastrointestinal leiomyosarcomas is only 21% (6). In this review, we present the results of chemotherapy for patients with disseminated gastric sarcoma referred to the M. D. Anderson Hospital and Tumor Institute for treatment, and we discuss our findings in light of experiences reported by other investigators.

MATERIALS AND METHODS

The records of our institute's tumor registry were reviewed in search of patients with primary sarcoma of the stomach treated with chemotherapy during the years 1945–1975. All patients included in this analysis had received chemotherapeutic agents after the diagnosis of metastatic sarcoma. Most patients had been referred to our institution for

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treatment after surgically unresectable recurrence of the neoplasm developed after curative resection of the primary tumor. The diagnosis for every patient was confirmed by our pathologists, who reviewed the slides and tissue blocks. In cases of equivocal diagnoses, a second biopsy was done at our hospital and an electron microscopic examination was performed to confirm the diagnoses. Prior to initiation of chemotherapy, most patients had a complete workup which included blood cell counts, renal and liver function tests, chest roentgenograms, and liver scans; some patients had angiographic evaluation of their abdominal tumor.

The chemotherapeutic agents used in the treatment of the patients included in this study varied according to the choice of the physicians treating the patients and the availability of the drugs. The intensity and duration of drug therapy varied from patient to patient, but most patients received treatments to their limits of tolerance and therapeutic benefit. The response to chemotherapy was determined by measuring the tumor size, based on the findings obtained from physical examinations, roentgenographic studies, or radioisotopic liver scans. A complete response was defined as a complete disappearance of all signs and symptoms related to the tumor and recovery of normal functions. A partial response (PR) was defined as a $\geq 50\%$ reduction in the sum of the products of the two largest perpendicular diameters of measurable tumor lesions without the appearance of new lesions. If hepatomegaly was the primary indicator of response, there must have been a reduction by at least 30% in the sum of liver measurements below each costal margin at the midclavicular lines and xiphoid process accompanied by improvement in liver function and liver scan. Stable disease was defined as a $< 50\%$ tumor regression or improvement in symptoms accompanied by no change in tumor size for at least 8 weeks from the start of treatment. Progressive disease was defined as a continued increase in tumor size or the appearance of new lesions.

RESULTS

Seventeen of 43 patients with primary leiomyosarcoma of the stomach seen at our institution during the 31 years under investigation received 23 trials of chemotherapy. No chemotherapy was utilized prior to 1959. The indication for chemotherapy was mainly symptomatic metastases, such as massive hepatomegaly or extra-abdominal metastases. The median age for the group was 52 years (range, 31–67 years). The metastatic tumor involved the liver in 13 patients, lung in three, bone in one, supraclavicular lymph nodes in one, and skin in one. Tumor recurrence in the gastric stump presenting as an epigastric mass was seen in four patients.

A patient was considered to have received an adequate trial if he received two courses of chemotherapy and survived for at least 3 weeks following the initiation of the second course. Most of the patients treated prior to 1970 received single-agent chemotherapy. DTIC was used for three patients, ADR for two, and a combination of both drugs for three. Chemotherapeutic agents frequently used in combination with ADR and DTIC were cyclophosphamide (CYC), vincristine (VCR), and actinomycin D (ACD).

The therapeutic effects of chemotherapy are summarized in table 1. Two patients had PRs and ten had stabilization of disease. Responses were seen in liver (one of 13 patients) and abdominal wall (one of one patient) metastases. One patient responded to ADR-DTIC and the other patient responded to these drugs given in combination with CYC and VCR. The responses lasted for 16 and 19 weeks, respectively. Stabilization of disease occurred in ten patients. The median duration of stable disease was 12 weeks (range, 9–17 weeks).

The median survival of the 17 patients from initiation of chemotherapy was 11 months. The two patients who had objective tumor regression lived

TABLE 1.—Chemotherapy for leiomyosarcoma of the stomach: M. D. Anderson Hospital experience

Treatment	No. of patient trials	No. of patients with—	
		PR	Stable disease
Single agents			
DTIC	3	—	1
ADR	2	—	2
CYC	1	—	—
Combination chemotherapy			
CYC+VCR+ADR+ACD	3	—	3
ADR+DTIC	3	1	1
CYC+VCR+ADR+DTIC	2	1	1
VCR+ADR+DTIC	2	—	—
CYC+ACD	2	—	1
CYC+VCR+ACD	1	—	1
CYC+methyl-CCNU	1	—	—
CYC+5-fluorouracil	1	—	—
ADR+methyl-CCNU	1	—	—
VCR+ACD+L-asparaginase	1	—	—

longer (16 and 24 months) than patients who had disease stabilization (median, 8 months; $P = 0.07$) or the patients who had progressive disease (median, 8 months; $P = 0.04$). There was no significant difference in survival time between patients who had disease stabilization and those who had disease progression.

DISCUSSION

Over the past few years, radical surgery alone and in combination with adjuvant radiotherapy or chemotherapy has been utilized to prevent recurrence of this malignant neoplasm (7–9). The failure of such multimodality treatments is usually due to the inability to control or prevent metastases, particularly in distant organs. In view of the rarity of sarcomas in general and of the gastric origin in particular, and despite the extensive literature on multi-drug chemotherapy for sarcomas (10–12), little is known about the response of disseminated gastric leiomyosarcoma to the different chemotherapeutic agents. In most studies of large series, no attempt has been made to correlate response with the site of the primary tumor or its histologic type. However, it has been reported that sarcomas of gastrointestinal origin are less responsive to chemotherapy than sarcomas arising from other sites (11).

Table 2 summarizes the literature on the experience with various chemotherapeutic agents used in the treatment of leiomyosarcomas of various primary sites. The majority of patients in these series had leiomyosarcoma of extragastrointestinal sites (in particular, of the genital organs). The responses obtained with ACD (13,14), VCR (15,16), and methotrexate (MTX) (17) usually were of short duration (average, 12–15 weeks) and without significant impact on the survival of the patients. The VCR-ACD-CYC combination regimen showed marked efficacy against soft tissue sarcomas (18). Two of 21 patients with sarcoma treated with this regimen had leiomyosarcoma and both patients had objective tumor regression.

TABLE 2.—Reported experience with chemotherapy for leiomyosarcomas

Treatment	No. of patients	No. of responders (%)	Ref No.
Single agents			
ADR	37	12 (32)	6
ACD	30	13 (43)	13,14
DTIC	24	6 (25)	11
MTX	11	3 (27)	17
VCR	7	2 (29)	15,16
Combination chemotherapy			
ADR+DTIC	38	19 (50)	6
CYC+VCR+ADR+DTIC	28	19 (68)	6
VCR+ADR+DTIC	22	8 (36)	6
CYC+ADR+ACD+DTIC	6	0 (0)	12
VCR+CYC+ACD	2	2 (100)	18

ADR administered alone or in combination with DTIC and other chemotherapeutic agents showed significant antitumor activity against sarcomas of various histologic types. The overall response rate was 46% (10), similar to the rate of 44% obtained in leiomyosarcomas of various primary sites. The response rate was only 21% for patients with sarcomas of gastrointestinal origin (mainly leiomyosarcomas) using the same chemotherapy combination (11); the response rate was only 15% for patients with gastric leiomyosarcoma in our series using the ADR-containing regimens.

The low rate of response to fairly intensive chemotherapy regimens administered to these patients with gastric leiomyosarcoma might be partially due to the frequent occurrence of liver metastases in patients with gastrointestinal tumors and the decreased responsiveness of the liver metastases to systemic chemotherapy. Since the liver is the most common site of recurrence of sarcoma, occurring in > 60% of the patients who had had curative surgery (19), and since the complete and overall response and survival rates are highly dependent on the dose of ADR administered (20), intrahepatic arterial administration of ADR to these patients might improve the rate of response of metastatic disease in this organ. Also, such a treatment could serve as effective adjuvant treatment for patients with a high risk of developing hepatic metastases. High-risk patients include those with primary leiomyosarcomas of the stomach measuring ≥ 8 cm, with tumor extension to serosa, and those with poorly differentiated malignancies (19). Intrahepatic chemotherapy has been found effective in reducing the appearance of liver metastases following resection of colorectal cancer (21).

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High-Pressure Liquid Chromatographic Analysis of 3-Deazauridine-5′-Triphosphate in Human Cancer Cells [1,2]

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SUMMARY

A rapid high-pressure liquid chromatographic assay for the detection and quantitation of 3-deazauridine-5′-triphosphate (deazaUTP), the active metabolite of the anticancer drug 3-deazauridine (deazaUrd), in cell extracts is described. This procedure permits the simultaneous detection and quantitation of CTP, the cellular concentration of which is affected by treatment with deazaUrd. Human lymphoblastoid cells (line CCRF-CEM) treated with 100 nmols of deazaUrd/ml in culture accumulate deazaUTP to > 25 nmols/10^7 cells after 4 hours. The concentration of CTP in these cells decreased exponentially with a half-life of 1.1 hours. After these cells had been incubated with 100 nmols of deazaUrd/ml and were resuspended in drug-free medium, the intracellular deazaUTP concentration decreased exponentially with a half-life of 3.4 hours. This assay has been applied to clinical studies of deazaUrd; high cellular deazaUTP concentrations have been detected in brain tumor tissue after deazaUrd infusion.

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The synthesis of 3-deazauridine (deazaUrd) was prompted by the biologic activity of other heterocyclic nucleosides with modified pyrimidine ring systems (1–3). DeazaUrd was shown to be toxic to bacteria (2) and cells in culture (2,4–6), to possess antiviral activity (7–9), and to elicit antitumor responses in murine systems (4,10). It is presently undergoing evaluation for anticancer activity in clinical trials (11,12).

DeazaUrd is phosphorylated to 3-deazauridine-5′-triphosphate (deazaUTP) (13,14), inhibiting CTP synthetase (EC 6.3.4.2), which aminates UTP to CTP (4,14). The findings that cytidine nucleotides are reduced in deazaUrd-treated cells (4) and that cytidine and deoxycytidine reverse the toxicity of deazaUrd (4,7,14) support this mechanism of deazaUrd action. Although 3-deazauridine-5′-diphosphate (deazaUDP) is inhibitory to the reduction of CDP to dCDP and deazaUrd-treated cells have lower deoxynucleotide pools than do control cells, this pathway is thought to be of secondary importance (4).

This report describes high-pressure liquid chromatographic techniques that permit the detection and quantitation of deazaUTP simultaneously with the analysis of normal cellular ribonucleotide triphosphate constituents in deazaUrd-treated human lymphoblastoid cells. The suitability of these techniques for clinical pharmacology is demonstrated by the detection of nucleotides in extracts of a metastatic brain tumor removed from a patient shortly after infusion of deazaUrd.

MATERIALS AND METHODS

A sample of deazaUrd (lot No. UI 75-107) was supplied by the Division of Cancer Treatment, Na-

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tional Cancer Institute, and [6-^{14}C]deazaUrd was kindly supplied by Drs. A. Bloch and P. Creaven of Roswell Park Memorial Institute. The radiochemical purity of this sample was determined to be 99% after elution from a column of μ-Bondapak C_{18} (Waters Associates) with a linear aqueous methanol gradient. Ribonucleotides and deoxyribonucleotides, used as standards in chromatographic separations, were purchased from Sigma Chemical Corp.

CCRF-CEM cells, originally cultured from the peripheral blood of a patient with acute lymphocytic leukemia (15), were obtained from Dr. E. Hersh of M. D. Anderson Hospital and Tumor Institute. The cells were grown in agitated suspension culture in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (Grand Island Biological Corp).

A 56-year-old man with renal cell carcinoma underwent craniotomy for removal of a solitary cerebral metastasis. As the dura was opened, a 30-minute infusion of deazaUrd (2.5 g/m^2) was begun. The tumor was resected 2.5 hours after the completion of the infusion and was prepared for nucleotide analysis as described below.

Nucleotide extracts were prepared in the following manner. After having been washed in phosphate-buffered saline (8.1 g of NaCl, 0.22 g of KCl, 1.14 g of Na_2HPO_4, and 0.27 g of KH_2PO_4 per liter of H_2O, pH 7.4), cells were resuspended and tumor was homogenized in 0.4 N perchloric acid. The acid-insoluble material was removed by centrifugation and re-extracted with one half the original volume of perchloric acid. After centrifugation, the acid-soluble supernatants were combined and neutralized with KOH, and the resulting $KClO_4$ was removed by centrifugation. The supernatants were stored at −30°C until analysis.

A Water Associates ALC 204 high-pressure liquid chromatograph equipped with two model 6000A pumps and a model 660 gradient programmer was used to fractionate acid-soluble extracts. Samples (0.10–2.0 ml) were injected onto a column of Partisil-10 SAX anion exchange resin (4.6 mm × 25 cm, Whatman Inc) with a model U6K injection system. They were fractionated by gradient elution using buffer A, 0.005 M $NH_4H_2PO_4$, pH 2.8, and buffer B, 0.750 M $NH_4H_2PO_4$, pH 3.7. Following isocratic elution at 40% buffer B for 10 minutes, a concentration gradient increasingly linearly to 100% buffer B was run over a 24-minute period at a flow rate of 2 ml/minute. Eluted compounds were detected at 280 nm by a model 440 detector (Waters Associates), and peak areas were quantitated with a CDS-111 electronic integrator (Varian Associates). Absorbance was recorded by a model 9126 (Varian Associates) 10-mv strip chart recorder. Peak areas were converted to absolute quantities using predetermined calibration curves. Before use, the Partisil column was purged with 50 ml of 2 M NH_2PO_4, pH 4.2, equilibrated with 40% buffer B and treated with 0.10 ml of 1.0 mM disodium ethylenediamine tetraacetate. Following a nucleotide analysis, the column was purged with 20 ml of 40% buffer B before injection of a subsequent sample.

The periodate oxidation procedure used in the identification of deazaUTP and cellular deoxynucleoside triphosphates was carried out by the method of Neu and Heppel (16). The method quantitatively removed ribonucleotides (< 0.1% remains) while deoxyribonucleotides and arabinosylnucleotides were unaffected (> 99% remains), as determined by analysis with high-pressure liquid chromatographic techniques. The details of this procedure will be reported separately.

RESULTS

The separation of 1 nmol of each ribonucleoside-5′-triphosphate by high-pressure liquid chromatography is shown in figure 1. Since ribonucleoside-5′-monophosphate and -5′-triphosphate are present at < 10% of the corresponding ribonucleoside-5′-triphosphates under the described growth conditions of CCRF-CEM cells, this procedure was designed to produce maximum resolution of the triphosphates in the shortest time.

Fractionation of the acid-soluble material from 2 × 10^6 CCRF-CEM cells is shown in figure 2. A comparison of peak areas with those of known quantities of authentic compounds indicated that 1.7 nmols of CTP, 4.1 nmols of UTP, 8.4 nmols of

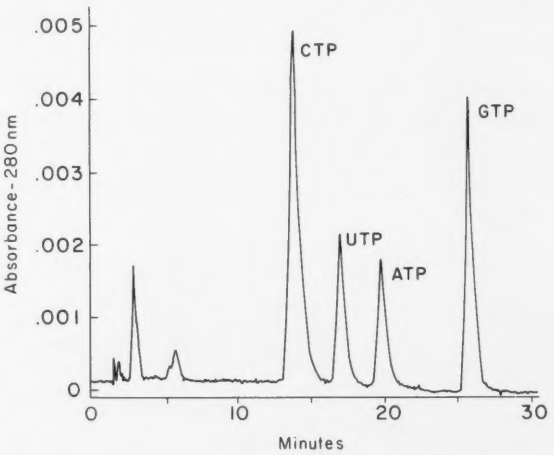

FIGURE 1.—Separation of 1 nmol each of CTP, UTP, ATP, and GTP by high-pressure liquid chromatography.

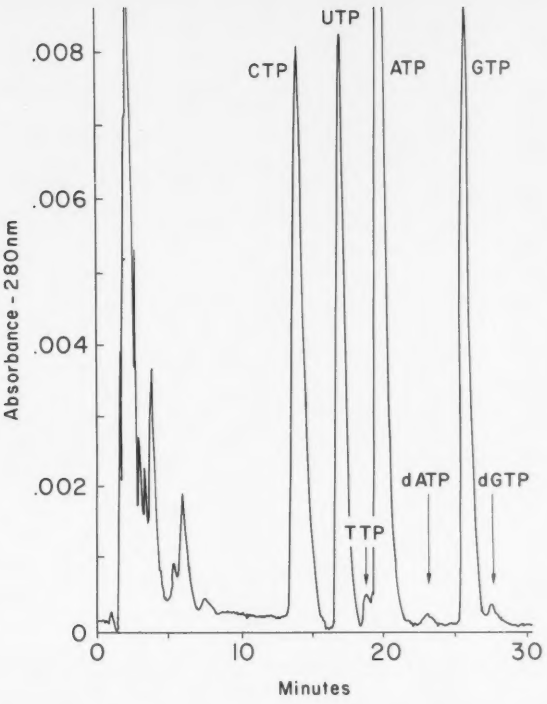

FIGURE 2.—Separation of PCA-soluble material extracted from the equivalent of 2 × 10^6 CCRF-CEM cells by high-pressure liquid chromatography.

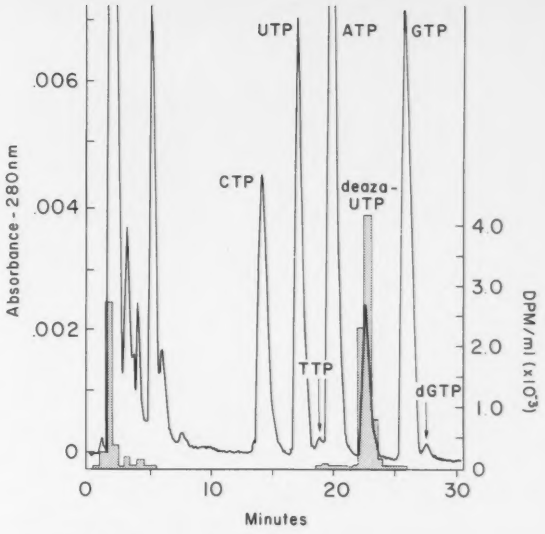

FIGURE 3.—High-pressure liquid chromatographic fractionation of PCA-soluble material extracted from the equivalent of 2 × 10^6 CCRF-CEM cells after incubation with 100 nmols of [^{14}C]deazaUrd/ml (specific activity, 1.16 × 10^6 dpm/μmol) for 2 hrs. The radioactivity in the column effluent was determined by liquid scintillation counting on fractions collected at 0.5-min intervals and is indicated by the dotted bar graphs.

ATP, and 2.3 nmols of GTP were present in the extract from these cells. In addition, this procedure permits reproducible separation and quantitation of both dATP and dGTP. Satisfactory resolution of TTP, shown in figure 2, is not as reproducible, whereas dCTP elutes on the trailing edge of CTP in this procedure and cannot be quantitated. The basis for assigning the identity to deoxynucleoside triphosphates was that they co-eluted with authentic compounds and they were stable to oxidation with $NaIO_4$.

After incubation with 100 nmols of [^{14}C]deazaUrd/ml for 2 hours, the cellular CTP levels had decreased, whereas those of UTP, ATP, and GTP were essentially unchanged (fig 3). In addition, a new peak eluting between ATP and GTP, with a retention time 0.5 minute less than that of dATP, was present. This peak had a greater absorbance at 280 nm than at 254 nm, was destroyed by $NaIO_4$, and was absent after incubation with phosphatase. Analysis of the column eluate for ^{14}C indicated that > 67% of the activity, equal to 6.4 nmols of deazaUrd, was associated with this peak. On the basis of these observations, this peak has been identified as deazaUTP.

Serial dilutions of the cell extract shown in figure 3 were fractionated by high-pressure liquid chromatography and the relationship between the peak area and the nmols of [^{14}C]deazaUTP is shown in figure 4. Although the peak area of dATP is included in the deazaUTP peak, it was assumed that, as in the case of ATP, deazaUrd treatment of the cells caused no significant change in cellular dATP levels. Subsequently, the peak area of dATP determined in the control cells (0.49 × 10^5 integrator units/2 × 10^6 cells; fig 2) was subtracted from the integrator units of deazaUTP in each serial dilution. After this small correction, the relationship between nmols of deazaUTP and the peak area is linear, with a practical lower limit of detection of about 0.25 nmol/sample.

Cells incubated continuously with 100 nmols of deazaUrd/ml rapidly accumulated deazaUTP to a plateau level of about 25 nmols of deazaUTP/10^7 cells by 4 hours (fig 5). Although this treatment had little effect on the cellular levels of UTP, ATP, or GTP (fig 6), the CTP level in these cells decreased exponentially with a half-life of 1.1 hours, suggesting an effective block in the synthesis of CTP. These effects of deazaUrd on normal cellular nucleotides are similar to those reported for L1210 cells (4,17).

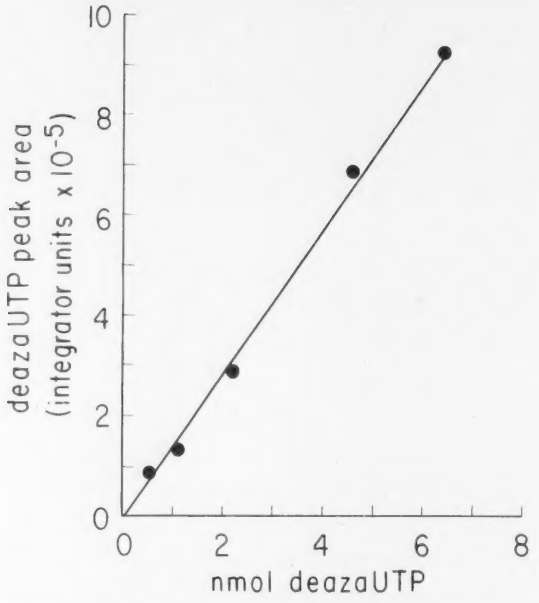

FIGURE 4.—Relationship between the deazaUTP peak area observed in dilutions of the extract described in fig 3 and the nmols of deazaUTP determined from the radioactivity that co-eluted with the deazaUTP peak.

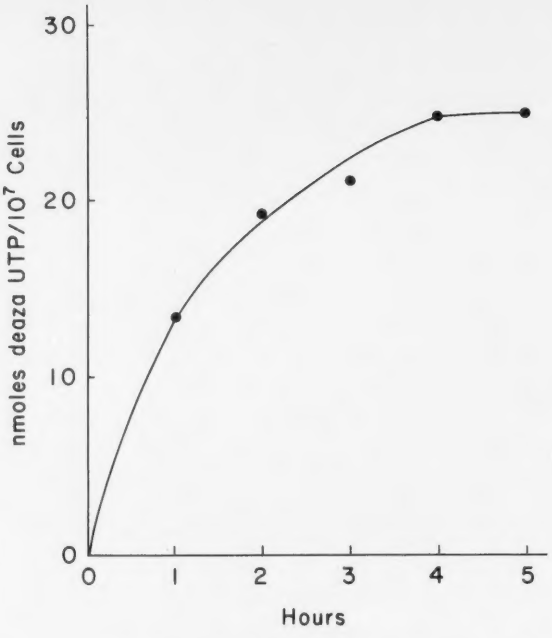

FIGURE 5.—Accumulation of deazaUTP by CCRF-CEM cells incubated with 100 nmols of deazaUrd/ml.

In a separate experiment, cells incubated for 4 hours with 100 nmols of deazaUrd/ml and then washed into drug-free medium lost deazaUTP with a half-life of 3.4 hours (fig 7). Thus, when the synthesis of either CTP or deazaUTP is inhibited, the subsequent rate of utilization of the normal nucleotide CTP is about three times greater than that of the antimetabolite deazaUTP.

The chromatographic technique for the detection and quantitation of deazaUTP is directly applicable to biochemical pharmacologic studies in patients receiving deazaUrd chemotherapy. Patients received an iv infusion of deazaUrd shortly before resection of a renal cell carcinoma metastatic to the brain. Chromatographic analysis of the acid-soluble material of this tumor is shown in figure 8. In addition to the four ribonucleoside-5′-triphosphates, deazaUTP was clearly present in this sample. By comparing the peak areas with those of known quantities of standards, it was determined that this sample from the equivalent of 55 mg of tumor contained the following quantities of nucleotide: CTP, 2.0 nmols; UTP, 7.0 nmols; ATP, 14.8 nmols; GTP, 5.0 nmols; and deazaUTP, 3.5 nmols. Making the conservative assumption that 1 g of tumor is contained in a volume of 1 ml, the concentration of

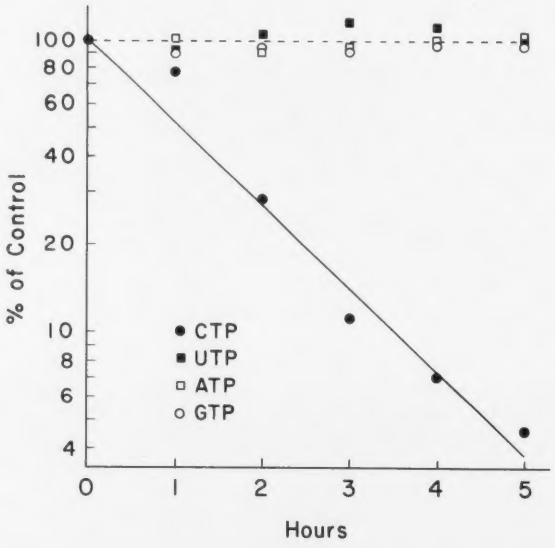

FIGURE 6.—Effect of 100 nmols of deazaUrd/ml on the levels of ribonucleotides in CCRF-CEM cells. Control levels of each nucleotide were: CTP, 5.5 μmols/10^7 cells; UTP, 22.0 μmols/10^7 cells; ATP, 43.9 μmols/10^7 cells; and GTP, 11.3 μmols/10^7 cells.

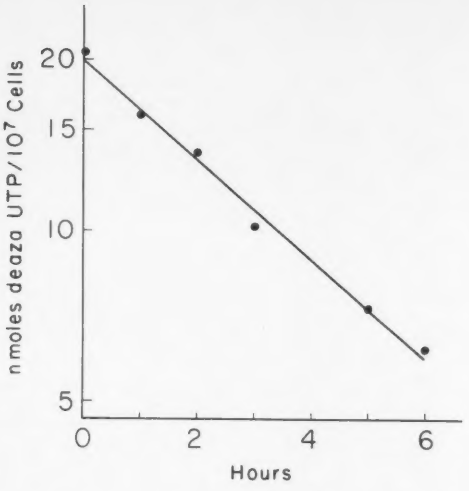

FIGURE 7.—Cellular levels of deazaUTP after a 4-hr incubation with 100 nmols of deazaUrd/ml and washing into drug-free medium.

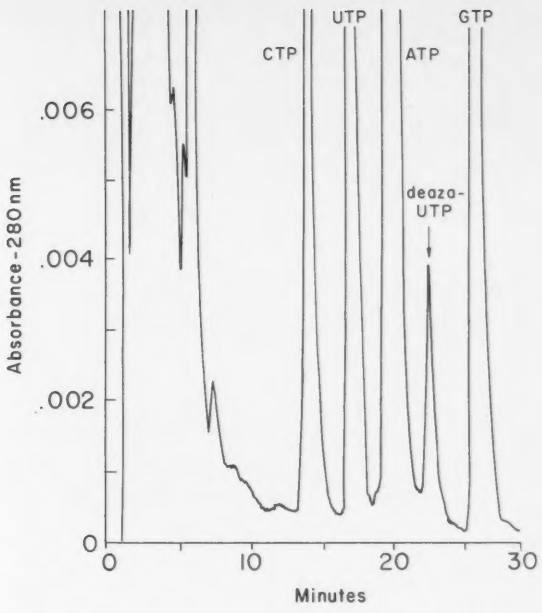

FIGURE 8.—High-pressure liquid chromatographic fractionation of PCA-soluble material extracted from the equivalent of 55 mg of brain tumor excised from a patient 2.5 hrs after iv infusion of 2.5 g of deazaUrd/m².

nucleotides in the tumor may be calculated as follows: CTP, 35 μM; UTP, 112 μM; ATP, 236 μM; GTP, 80 μM; and deazaUTP, 62 μM. The latter figure demonstrates that deazaUrd not only entered the tumor and was phosphorylated to deazaUTP, but, more importantly, that this active nucleotide form of the drug accumulated to concentrations at least 12 times greater than the K_i of this compound for its target enzyme (~5 μM; ref 14).

DISCUSSION

We have described a high-pressure liquid chromatographic technique for the detection and quantitation of deazaUTP, the active form of the anticancer drug deazaUrd, in human cells in vitro and in a tumor preparation excised after deazaUrd infusion. Using the same technique, it is possible to determine simultaneously the level of CTP, the affected nucleotide, in cell extracts. The practical limits of sensitivity for deazaUTP and CTP by these procedures are 250 and 50 pmols respectively. Thus, measurements of the fluctuations in the cellular concentration of CTP also may serve as a sensitive indicator of the inhibitory action of deazaUTP.

We have found that other high-pressure liquid chromatographic elution schemes designed for the fractionation of nucleoside monophosphates, diphosphates, and triphosphates (18) or nucleoside diphosphates and triphosphates (19) provide adequate, although incomplete, separation of deazaUTP from other ribonucleotides. However, these procedures can separate deazaUDP, which is not possible with the method described herein. These procedures might have application to studies of deazaUrd phosphorylation by murine tumors in which significant levels of deazaUDP do accumulate (20). As mentioned earlier and shown in figure 3, human cells in culture and extracts of tumors as well as bone marrow specimens (manuscript in preparation) from patients treated with deazaUrd accumulate nucleotide metabolites predominantly as deazaUTP. These findings have influenced our decision to bypass routine analysis for the diphosphate in favor of a clear separation of deazaUTP in these tissues.

This rapid and sensitive assay for the active metabolite deazaUTP and the affected metabolite CTP should complement recently described assays for deazaUrd (21) in clinical studies of the biochemical pharmacology of this promising anticancer drug.

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Phase II Study of Leo 1031 (Prednimustine) in Advanced Ovarian Carcinoma [1]

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SUMMARY

Thirty-six patients with advanced ovarian carcinoma, 21 who had not responded to conventional surgery and irradiation therapy and 15 who had not responded to conventional surgery, irradiation therapy, and chemotherapy, were treated in a phase II study with Leo 1031. Objective responses were obtained in ten (28%) of the 36 patients (two complete and eight partial responses). An additional 11 (32%) patients had stable disease. The two complete responders were in remission for 4 months. During remission, all of the patients had an improved quality of life. Myelosuppression of varying severity occurred in 11 patients; three of these patients died from their complications. Two patients developed reversible confusion and one patient became psychotic. Eight patients became euphoric during treatment. Leo 1031 might be worthwhile as a palliative chemotherapy for patients with advanced ovarian carcinoma, but the risk of euphoria must always be considered since it can lead to voluntary overdosage.

[Cancer Treat Rep 63:421–424, 1979]

Ovarian carcinoma is the fifth most common female tumor in Sweden, and its frequency is continually increasing. Every year > 900 new cases are diagnosed (1). Approximately 70% of the patients initially have metastases outside the pelvic cavity and cannot be treated by curative surgery and/or radiation therapy. Most of these women will die as a result of their malignancy within the first 2 years after diagnosis (2).

The overall 5-year survival rate has, furthermore, shown little improvement during the past 30 years. In the hope of attaining better results, a considerable interest in chemotherapy has developed during the past 10 years.

Chemotherapy has previously been confined to those patients in whom the surgical and radiotherapeutic possibilities have been exhausted. Nonetheless, cytotoxic chemotherapy for advanced ovarian carcinoma has a well-established effect (3–5). Used as single drugs, the most effective drugs are probably the alkylating agents, with a response rate of 12%–64% (2,3,6). Among the antibiotics, adriamycin has shown a promising effect in a phase II study and the same range of effectiveness as melphalan in a randomized study (7,8). Although the antimetabolites, 5-fluorouracil and methotrexate, have mostly been used as second-line treatment, a response rate of about 25% has been recorded (6,9). Recently, two new drugs, hexamethylmelamine and *cis*-dichlorodiammineplatinum(II), have shown a high degree of efficacy, but also rather troublesome toxicity (10,11).

However, in spite of the initially good response rate seen with single agents and combination chemotherapy, the 5-year survival rates are very depressing. There is a need for clinical research investigating new drugs and new combinations of drugs. Leo 1031 (prednimustine), a chlorambucil ester of prednisolone, has proved to be effective in several experimental tumors (12). In initial clinical studies, Leo 1031 has shown encouraging results in a wide range of tumors: non-Hodgkin's lymphoma (13–15), breast cancer (16), chronic lymphatic and acute myelocytic leukemia (17), prostatic cancer (18),

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and malignant melanoma.[3] These results and the low rate of side effects reported prompted us to perform a phase II study in advanced recurrent ovarian carcinoma.

MATERIALS AND METHODS

Thirty-six patients with recurrent malignant tumors fulfilling the following criteria were accepted: leukocyte count $\geq 3.0 \times 10^9$/liter, platelet count $\geq 100 \times 10^9$/liter, and measurable or evaluable lesions. All patients were evaluable. The median age was 61 years (range, 44–81 years).

The patients were divided into two groups according to previous treatment: group A (table 1) had previously been treated by surgery and/or irradiation while group B (table 1), in addition to receiving surgery and/or irradiation, had also received chemotherapy for relapse (nine had received adriamycin plus melphalan and six had received melphalan). The median duration of remissions by previous therapy was 24 months (range, 0–144 months) in group A (16 of 21 patients) and 15 months (range, 7–216 months) in group B (15 of 15 patients). Histology revealed that 18 patients had serous cystadenocarcinoma, five had mucinous cystadenocarcinoma, seven had endometrioid carcinoma, two had granulosa cell carcinoma (hormone production not detectable), and four had poorly differentiated tumor.

Leo 1031 was given orally at a dose of 50 mg/m^2 divided in four daily doses. Therapy was administered in three induction courses. The first course lasted 28 days. After a rest period of 14 days, two more courses were given; each course lasted 14 days with a rest period of 14 days. Patients in remission after this induction therapy received maintenance therapy with 7 days of treatment every 5 weeks until a relapse was detected. A reduction in dose was made if myelosuppression occurred.

The results were defined as follows. Complete remission (CR) was a total disappearance of all measurable and evaluable lesions for at least 1 month. Partial remission (PR) was a $\geq 50\%$ reduction in the largest diameter of measurable tumor and total regression of evaluable, but nonmeasurable, lesions such as pleural or ascitic fluid. Stable disease was a $< 50\%$ decrease in the diameter of measurable lesions or no change in evaluable, but nonmeasurable, lesions, and the appearance of no new lesions. Ascitic and pleural fluids might persist, but an increase in the intervals between thoracocentesis and laparocentesis must have occurred. Progressive disease was an increase in tumor size or the appearance of new lesions during the treatment period without previous remission or a greater need for laparocentesis. A relapse was a new lesion, a recurrence, or an increase in remaining tumor in a previously regressing area after a remission.

TABLE 1.—Response to Leo 1031 therapy

Patient No.	Response*	Duration (mos) of—	
		Remission	Survival
	Group A		
1	PR	20+	22+
2	PR	2	13+
3	PR	4	21+
4	SD	3	17
5	SD	2	4
6	SD	10	22+
7	PD	—	8
8	PD	—	13
9	PD	—	14
10	PD	—	7
11	PD	—	16+
12	PR	18	39+
13	SD	4	11
14	CR	4	14
15	PR	7	20
16	PR	2	3
17	SD	4	8
18	SD	3	7
19	CR	4	22
20	PD	—	3
21	PD	—	14
	Group B		
22	SD	13	20+
23	PD	—	3
24	PD	—	8
25	PD	—	5
26	PD	—	4
27	PD	—	2
28	PD	—	4
29	PR	6	13
30	PR	6+	8+
31	SD	2	3
32	PD	—	5
33	PD	—	7
34	SD	3	5
35	SD	3	6+
36	SD	2	7

*SD = stable disease and PD = progressive disease.

RESULTS

Objective response was obtained in ten (28%) of the 36 patients (two CRs and eight PRs) (table 1). Furthermore, 11 patients (32%) had stable disease for a median duration of 3 months (range, 2–13 months). The two CRs lasted for 4 months. The median duration for PRs was 6 months (range, 2–22+ months). The median survival was 14 months (range, 3–31 months) for responders, 7 months

[3]European Organization for Research on Treatment of Cancer. Early clinical trial: general protocol for co-operative chemotherapy screening study in human cancer, 1973.

(range, 3–22+ months) for patients with stable disease, and 7 months (range, 2–14 months) for nonresponders (table 1). Thus, in group A responses occurred in three of 11 patients with serous cystadenocarcinoma, one of two with mucinous cystadenocarcinoma, three of five with endometrioid carcinoma, and one of three with poorly differentiated carcinoma. In group B, PRs were observed in two patients with mucinous carcinoma.

The overall objective response rate was 38% (eight of 21 patients) for the patients who had not had previous chemotherapy compared with 13% (two of 15 patients) who had received previous chemotherapy. Three of four patients with tumor limited to the pelvis had an objective remission compared with seven of 32 patients with more advanced tumors.

All of the responding patients had a better quality of life during their remissions, and the median improvement in the Karnofsky performance index was 20 points (range, 0–60 points). Ten of 11 patients with stable disease had an improved general condition, and the median improvement in the Karnofsky index was 10 points (range, 0–40 points). The interval between the start of treatment and the assessment of objective response was generally < 2 months.

Side Effects

Four patients complained of transient nausea and gastritis. Two patients developed reversible confusion and one patient became psychotic. When the treatment was withdrawn, this patient was restored to a normal mental condition. Eight patients became euphoric during treatment. Eleven patients had a nadir value of leukocytes $< 2.0 \times 10^9$/liter, and ten had a nadir value of platelets $< 50 \times 10^9$/liter. Three of these patients developed severe secondary infections and bleeding which resulted in death. In the other patients, the leukopenia and thrombocytopenia were reversible. The blood cell counts were normalized in 2–6 weeks. No other side effects were observed.

DISCUSSION

This study shows that Leo 1031 can induce remissions in ovarian carcinoma. The overall response rate in patients who had not previously been treated with chemotherapy was 38%, which is comparable with that achieved with alkylating agents (2,3,6), adriamycin (7,8), antimetabolites (6,9), *cis*-dichlorodiammineplatinum(II) (11), and hexamethylmelamine (10).

As second-line chemotherapy, Leo 1031 showed objective response in two of 15 patients. This means that Leo 1031 is not totally cross-resistant to alkylating agents since these two responders had been previously exposed to melphalan. Both of these patients had mucinous carcinoma.

In the present series, eight of 21 patients responded to chemotherapy after previous irradiation which favorably compares to the data of Piver et al (2) who saw no responses with melphalan in 22 similar patients.

In previous studies only mild side effects were observed. Toxicity was expressed by occasional mental disturbances, weak cushingoid symptoms, gastrointestinal intolerance, and mainly myelosuppression, but no drug-related deaths. In the present trial, a rather high rate of side effects was noticed. Three drug-related deaths occurred in patients with secondary infections and bleeding due to myelosuppression. One of the patients voluntarily took overdoses of the drug because she had euphoria. This feeling of well-being was also observed in seven other patients. The mental effect of Leo 1031 is probably caused by the prednisolone factor and might make the drug useful as a palliative treatment; however, this effect is troublesome since a risk of developing confusion exists. All of the patients who had myelosuppression were previously irradiated and four of them had also received chemotherapy, which is an important contributory factor to the tolerance of chemotherapy. Another possible explanation for the side effects is the dose and the schedule used, which are somewhat more intensive than in previous studies, particularly considering the poor general condition of the patients and the prior therapy.

In our opinion, Leo 1031 might be worthwhile as palliative chemotherapy for patients with ovarian carcinoma. We are now testing Leo 1031 in different schedules, comparing low-dose maintenance to high-dose intermittent therapy.

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Experimental Antitumor Activity of Aminoanthraquinones [1,2]

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SUMMARY

The activity of a number of substituted alkylaminoanthraquinones was compared in transplanted murine tumor systems including P388 and L1210 leukemias, B16 melanoma, and colon carcinoma 26. The structure-activity relationships among this class of compounds are discussed. Several derivatives had very high antitumor activity in several tumor systems. Two of the most active derivatives, namely, 1,4-bis{2-[(2-hydroxyethyl)amino]ethylamino}-9,10-anthracenedione (bisalkylAAD) and 1,4-dihydroxy-5,8-bis{2-[(2-hydroxyethyl)amino]ethylamino}-9,10-anthracenedione (dihydroxybisalkylAAD), which had curative activity in the above-mentioned tumors, were compared in considerable detail. DihydroxybisalkylAAD showed distinct advantages over bisalkylAAD in several tumor systems and is tenfold more potent with respect to effective dose range. This last difference is important for two reasons. First, these aminoanthraquinones are strong and persistent blue dyes and the administration of lower doses would minimize a potential cosmetic drawback of these compounds. Second and most important, iv administration of dose levels of bisalkylAAD which are within the therapeutic dose range on intermittent dose schedules produced convulsions and immediate death. Iv administration of dihydroxybisalkylAAD also caused acute toxicity, but, because of its increased potency relative to antitumor activity and delayed toxicity, this acute toxicity was apparent only at doses well above the therapeutic dose range. All of the aminoanthraquinones evaluated, regardless of their activity as antitumor agents in vivo, proved to be potent inhibitors of DNA and RNA synthesis in vitro and bound strongly to DNA as evidenced by ΔTm values (ΔTm = upward shift in DNA melting temperature). Thus, the strong antitumor activity of aminoanthraquinones would appear to be due to some mechanism other than, or in addition to, DNA binding and inhibition of nucleic acid synthesis.

[Cancer Treat Rep 63:425–439, 1979]

Recently, the synthesis and antitumor activity of a series of bis(substituted aminoalkylamino)-anthraquinones were described (1). The original active agent of this series, 1,4-bis{2-[(2-hydroxyethyl)amino]ethylamino}-9,10-anthracenedione (bisalkylAAD), was discovered by random screening in the National Cancer Institute (NCI) program and led to the synthesis of a number of analogs. The structures of bisalkylAAD [*1*] and 19 analogs, a

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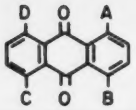


Compound	NSC number	Substituent			
		A	B	C	D
1	196473 287513	$-NH(CH_2)_2\ NH(CH_2)_2\ OH$	same as A	-H	-H
2	279836 299195	$-NH(CH_2)_2\ NH(CH_2)_2\ OH$	same as A	-OH	-OH
3	299187	$-NH(CH_2)_2\ NH(CH_2)_2\ OH$	-OH	-H	-H
4	276740	$-NH(CH_2)_2\ NHCH_2CH_3$	same as A	-H	-H
5	291923	$-NH(CH_2)_2\ NHCH_3$	same as A	-H	-H
6	281246	$-NH(CH_2)_2\ NH_2$	same as A	-H	-H
7	300576	$-NH(CH_2)_2\ N(CH_3)_2$	same as A	-H	-H
8	300579	$-NH(CH_2)_2\ N(CH_3)_2$	-OH	-H	-H
9	295561	$-NH(CH_2)_2\ N(CH_3)_2$	-H	same as A	-H
10	278467	$-NH(CH_2)_3\ NH(CH_2)_2\ OH$	same as A	-H	-H
11	294726	$-NH(CH_2)_3\ N(CH_3)_2$	same as A	-H	-H
12	293844	$-NH(CH_2)_3\ N(CH_3)_2$	-H	same as A	-H
13	294725	$-NH(CH_2)_3\ N(CH_3)_2$	-H	-H	same as A
14	291924	$-NH(CH_2)_2\ NH(CH_2)_2\ CH_3$	same as A	-H	-H
15	279837	$-NH(CH_2)_2\ N\bigcirc NH$	same as A	-H	-H
16	279838	$-NH(CH_2)_2\ N\bigcirc$	same as A	-H	-H
17	281249	$-NH(CH_2)_2\ NH(CH_2)_2 NH(CH_2)_2\ OH$	same as A	-H	-H
18	285685	$-NH(CH_2)_5\ OH$	same as A	-H	-H
19	285688	$-NH(CH_2)_2\ S(CH_2)_2\ OH$	same as A	-H	-H
20	208738	$-NH(CH_2)_3\ N(CH_3)_2$	-OH	-H	-H

FIGURE 1.—Structures and NSC Nos. of aminoanthraquinone derivatives.

number of which have extensive activity against transplantable rodent tumors, are shown in figure 1. It is the objective of this report to describe the activity of this class of compounds, expand upon previously reported (1) structure-activity relationships, and present evidence that one of the analogs, namely, 1,4-dihydroxy-5,8,bis{2-[2-hydroxyethyl)-amino]ethylamino}-9,10-anthracenedione (dihydroxybisalkylAAD, [*2*]), possesses therapeutic, toxicologic, and pharmacologic characteristics which make it the most attractive candidate among these aminoanthraquinones for development toward clinical trial.

MATERIALS AND METHODS

Drugs

The synthesis of 12 of the compounds included in this study was reported by Zee-Cheng and Cheng (1). The synthesis of the remaining eight compounds is described briefly in table 1.

For administration to animals, the compounds which were in the free base form (compounds [*1*] [bisalkylAAD], [*2*] [dihydroxybisalkylAAD], [*3*]–[*8*], [*10*], and [*14*]–[*17*]) were dissolved in 0.9% NaCl solution by the addition of one or two molar equivalents of HCl (depending on the number of basic nitrogen atoms). Compounds [*18*] and [*19*], which have no basic nitrogen in the side chains, were administered as suspensions in 0.3% hydroxypropylcellulose-0.9% NaCl solution. The remaining compounds which were in the form of diacetate or dihydrochloride salts were dissolved in 0.9% NaCl solution. Either the free bases or diacetate salts of compounds [*1*] and [*2*] were used in the various chemotherapy trials; data are not presented individually for the two forms of these compounds since their activities were identical. Drug solutions were diluted to an appropriate concentration so that the desired dose was delivered in a volume of 0.01 ml/g of body weight.

Mice and Tumors

For chemotherapy experiments, protocols described by Geran et al (5) were followed. DBA/2,

TABLE 1.—Synthesis of aminoanthraquinone derivatives*

Compound	Preparation		Formula	Analysis‡	Melting point (°C)
	Method†	% yield			
[3]	A	85	$C_{18}H_{16}N_2O_4$	C,H,N	138–140
[7]	A	87	$C_{22}H_{28}N_4O_2$	C,H,N	170–172
[8]	A	36	$C_{18}H_{16}N_2O_3 \cdot 0.25\ H_2O$	C,H,N	118–120
[9]	B	72	$C_{22}H_{28}N_4O_2 \cdot 2\ HCl \cdot 0.5\ H_2O$	C,H,N,Cl	> 300
[11]	A	25	$C_{24}H_{32}N_4O_2 \cdot 3\ HCl \cdot 0.2\ H_2O$	C,H,N,Cl	> 300
[12]	B	64	$C_{24}H_{32}N_4O_2 \cdot 2.2\ HCl \cdot 1.2\ H_2O$	C,H,N,Cl	> 300
[13]	B	58	$C_{24}H_{32}N_4O_2 \cdot 2.1\ HCl \cdot 0.8\ H_2O$	C,H,N,Cl	> 300
[20]	C	6.5	$C_{19}H_{20}N_2O_3 \cdot HCl \cdot H_2O$	C,H,N,Cl	> 300

* We are indebted to Edward W. Grange for synthesis of compounds [9], [11], [12], and [13], and to Robert B. Bicknell for preparation of compound [20].

† A = reaction of leucoquinizarin with excess alkylamine at 50°C for 1 hr under N_2, then air is passed through at 50°C for 45 mins following the procedure of Greenhalgh and Hughes (2). B = reaction of appropriate 1,x-dihaloanthraquinone in excess alkylamine at reflux for 3 hrs following the procedure of Double and Brown (3). C = reaction of quinizarin with excess 3-(dimethylamino)propylamine in pyridine at 40°C overnight following the procedure of Fokin et al (4). These authors prepared a series of 2-(alkylamino)quinizarin derivatives. Consequently, we expected the product of this reaction to be 2-[3-(dimethylamino)propylamino]quinizarin, but mass spectral data indicated the molecular ion to be that of compound [20].

‡ All of the compounds gave satisfactory analysis (within ± 0.4% of the calculated values) for the elements listed. In addition, all compounds exhibited the correct molecular ion (for the free base) by mass spectrometry.

C57BL/6, BALB/c, CD2F$_1$[(BALB/c × DBA/2)F$_1$], and B6D2F$_1$ [(C57BL/6 × DBA/2)F$_1$] mice of both sexes, weighing 17–23 g, were obtained from the Mammalian Genetics and Animal Production Section, Developmental Therapeutics Program, Division of Cancer Treatment, NCI. The following tumors were maintained by serial transplantation in syngeneic hosts and implanted in appropriate F$_1$ hybrids for chemotherapy trials: L1210 leukemia, P388 leukemia, sublines of P388 leukemia resistant to adriamycin or daunorubicin (6), B16 melanoma, colon carcinomas 26 and 38, M5076 ovarian carcinoma, Lewis lung carcinoma, and glioma 26.

Effects of Analogs on Thermal Denaturation of DNA

The influence of aminoanthraquinone derivatives and some known DNA intercalators on the melting temperature of calf thymus DNA was determined as described by Tong et al (7). The concentrations of drug and DNA were 5 and 50 μM respectively in 0.01 M phosphate buffer, pH 7, containing 10 μM EDTA and 5% DMSO.

Inhibition of Thymidine and Uridine Incorporation in Cultured L1210 Cells

L1210 cells were grown in RPMI–1640 medium containing 10% heat-inactivated fetal calf serum, 20 mM HEPES buffer at pH 7.2, 100 units of penicillin, and 100 μg of streptomycin per ml, and were maintained at a density of 1–4 × 10^6 cells/ml. Compounds were dissolved in DMSO and diluted with growth medium to yield a final DMSO concentration of 2%. Typically, drugs were assayed at final concentrations of 100, 30, 10, 3, 1, 0.3, and 0.1 μM. A second assay was performed at more closely spaced concentrations bracketing the inhibitory concentration range. All assays included a standard of daunorubicin as well as untreated cells. One-half ml of L1210 cells (2 × 10^6 cells/ml) was added to 0.5-ml dilutions of the drugs and incubated for 3 hours at 37°C in a shaking water bath. At this time, methyl-^{3}H-thymidine or 6-^{3}H-uridine (0.5 microcurie/12.5 nmols/ml) was added and cell suspensions were incubated for an additional hour. Incorporation of labeled precursors into TCA-insoluble material was determined as previously described (8).

RESULTS

Comparative Activity of Aminoanthraquinone Derivatives in Transplantable Tumor Systems

Comparative testing of aminoanthraquinone derivatives was performed in several ip transplanted tumor systems: P388 and L1210 leukemias, B16 melanoma, colon carcinoma 26, and anthracycline-resistant sublines of P388 leukemia. In these studies

TABLE 2.—Activity of aminoanthraquinone derivatives against ip P388 leukemia

Compound*	No. of experiments†	Maximally tolerated (optimal) dose range (mg/kg/day, ip, Days 1–9)	Average maximum ILS‡ (%)	Long-term survivors at optimal dose§/ No. treated
[1]	10	10–20	> 200	30/66
[2]	7	0.75–1.5	> 200	29/48
[3]	2	10–20	> 200	5/12
[4]	2	10–20	64	1/14
[5]	2	6–12	82	3/14
[6]	3	10–20	117	1/20
[7]	1	40	110	0/6
[8]	1	80	55	0/6
[9]	1	15	0	0/6
[10]	3	30–60	33	0/18
[11]	2	25–50	4	0/12
[12]	2	40–80	0	0/12
[13]	2	20–40	2	0/12
[14]	2	16–32	20	0/14
[15]	3	20–40	32	0/20
[16]	3	50–100	12	0/20
[17]	4	50–100	12	0/26
[18]	2	150–300	6	0/14
[19]	2	> 256	3	0/14
[20]	2	100–200	26	0/12

*See fig 1.

†Each experiment consisted of groups of 6 or 8 $CD2F_1$ mice treated with 5 logarithmically spaced dose levels of the individual agents administered ip on Days 1–9.

‡ILS at optimal dose relative to untreated controls.

§Tumor-free survivors 45 days after tumor implantation.

the drugs were administered ip on Days 1–9 and derivatives were always tested in comparison with the parent bisalkylAAD [1].

The results of a number of trials against P388 leukemia are summarized in table 2. The parent compound, [1], at its optimal dose of 10–20 mg/kg/day produced about 45% long-term survivors and showed an increase in lifespan (ILS) of > 200%. Two derivatives, [2] and [3], had similar high activity against P388 leukemia, with 40%–60% long-term survivors. Compound [2] proved to be about tenfold more potent than the parent compound in that the maximally tolerated dose was considerably less than that of the parent compound. Compounds [4]–[8] also had good activity against P388 leukemia, producing > 50% ILS with occasional long-term survivors for three of these compounds. Marginal activity was seen for compounds [10], [15], and [20]. The rest of the derivatives were devoid of activity even though several of them proved to be toxic to mice at dose levels similar to or slightly higher than the maximally tolerated doses of the active derivatives.

The results of comparative trials in mice bearing

TABLE 3.—Activity of aminoanthraquinone derivatives against ip L1210 leukemia*

Compound	No. of experiments	Maximally tolerated (optimal) dose range (mg/kg/day, ip, Days 1–9)	Average maximum ILS (%)	Long-term survivors at optimal dose†/ No. treated
[1]	5	8–16	123	12/32
[2]	3	0.75–1.5	178	8/20
[3]	1	7.5	56	1/6
[4]	2	16–32	36	0/12
[5]	2	8–16	94	1/12
[6]	2	8–16	246	5/12
[7]	1	30	39	0/6
[8]	1	160	33	0/6
[9]	1	30	0	0/6
[10]	1	50	0	0/6
[14]	1	16	12	0/6
[15]	1	32	12	0/6
[16]	1	64	12	0/6
[17]	1	32	12	0/6
[18]	1	> 256	12	0/6
[19]	1	> 256	6	0/6
[20]	1	100	0	0/6

*See footnotes in table 2.

†Tumor-free survivors 30 days after tumor implantation.

L1210 leukemia (table 3) were quite similar to the results in P388 leukemia. The parent compound, [1], and its dihydroxy derivative, [2], produced about 40% long-term survivors. In three direct comparisons, compound [2] increased lifespan to a greater extent than compound [1]: 162% versus 100%, 233% versus 106%, and 139% versus 89%. Compound [6] was also highly active against L1210 leukemia. The apparent high activity of compound [6] was a reflection of curative activity in one of two experiments. In the second experiment, compound [6] increased lifespan by 173% with no cures. Compounds [3], [4], [5], [7], and [8], which were active against P388 leukemia, also proved to be active (> 30% ILS) against L1210 leukemia. Compounds with marginal activity against P388 leukemia were inactive in the L1210 system, and P388-inactive agents were also ineffective against L1210 leukemia.

The aminoanthraquinones with extensive activity against the leukemias were also highly active against B16 melanoma as shown in the two experiments summarized in table 4. The parent compound, [1], and derivatives [2] and [3] produced > 50% long-term survivors in this solid tumor system. Compounds [5] and [6] were also quite active with > 100% ILS. Compounds [4], [7], and [8] had a low level of activity (about 50% ILS). The compounds with marginal or no activity against the leukemias were inactive in the B16 system.

The active aminoanthraquinones were evaluated against another solid tumor system, colon carci-

TABLE 4.—Activity of aminoanthraquinone derivatives against ip B16 melanoma

Compound*	% ILS (long-term survivors/No. treated) at optimal dose†	
	Experiment I‡	Experiment II‡
[1]	> 160 (7/10) at 16	> 233 (6/10) at 20
[2]	> 160 (8/10) at 0.5	> 233 (5/10) at 0.75
[3]	—	> 233 (6/10) at 30
[4]	54 (1/10) at 8	—
[5]	122 (2/10) at 8	—
[6]	109 (4/10) at 8	—
[7]	—	50 (0/10) at 40
[8]	—	58 (0/10) at 200
[9]	—	28 (0/10) at 10
[10]	—	6 (0/10) at 50
[14]	0 (0/10) at 32	—
[15]	2 (0/10) at 32	—
[16]	28 (1/10) at 32	—
[17]	0 (0/10) at 64	—
[18]	0 (0/10) at 128	—
[19]	0 (0/10) at 256	—

*See fig 1.

†Groups of 10 B6D2F$_1$ mice bearing ip B16 melanoma were treated ip on Days 1–9 at 5 logarithmically spaced dose levels of drugs. Long-term survivors 60 days after tumor implantation. Optimal dose is in mg/kg/day.

‡Median survival time of untreated controls (60 or 70 per experiment) was 23 days in experiment I and 18 days in experiment II.

TABLE 5.—Activity of aminoanthraquinone derivatives against ip colon carcinoma 26

Compound*	% ILS (long-term survivors/No. treated) at optimal dose†	
	Experiment I‡	Experiment II‡
[1]	172 (3/10) at 21	102 (4/10) at 10
[2]	> 275 (8/10) at 1	> 188 (5/10) at 0.75
[3]	—	88 (4/10) at 7.5
[4]	66 (0/10) at 16	—
[5]	131 (2/10) at 8	—
[6]	131 (2/10) at 16	—
[7]	—	4 (0/10) at 40
[8]	—	33 (0/10) at 40

*See fig 1.

†Groups of 10 CD2F$_1$ mice bearing ip colon carcinoma 26 were treated ip on Days 1–9 at 5 logarithmically spaced dose levels of drugs. Long-term survivors 75 days after tumor implantation. Optimal dose is in mg/kg/day.

‡Median survival time of untreated controls (50 or 60 per experiment) was 16 days in experiment I and 26 days in experiment II.

noma 26, with the results shown in table 5. The parent compound, [1], was also highly active in this tumor system, increasing lifespan by > 100% and producing 30%–40% long-term survivors. The dihydroxy derivative, [2], was reproducibly more active than the parent compound in colon carcinoma 26, producing > 50% long-term survivors at the optimal dose. Compounds [3], [4], and [6] were also quite active and compound [4], as in the other tumor systems, was less active against colon carcinoma 26 than the aforementioned derivatives. Compound [7], which was active against the other three tumors, was ineffective in colon carcinoma 26. Compound [8] had marginal activity, as in the other systems.

Because of the structural similarity of active aminoanthraquinones and anthracycline antibiotics (both possessing a planar quinoid ring system with a basic amino group in a side chain) and the evidence presented herein suggesting that the aminoanthraquinones intercalate into double helical DNA, several active derivatives were tested against sublines of P388 resistant to adriamycin and daunorubicin (table 6). The results show that both anthracycline-resistant lines were completely resistant to adriamycin and to the aminoanthraquinone derivatives [1], [4], [5], and [6]. The dihydroxy derivative, [2], retained some activity against these sublines, although partial cross-resistance is clearly evident. This result has been shown a number of times. The anthracycline-resistant sublines retained sensitivity to mitomycin C which was included as a positive control for the resistant sublines in these experiments.

Detailed Comparison of the Antitumor Activity of BisalkylAAD [1] and DihydroxybisalkylAAD [2]

The studies in L1210 leukemia, colon carcinoma 26, and the anthracycline-resistant sublines of P388 leukemia indicated that, in these systems, dihydroxybisalkylAAD has somewhat greater antitumor activity than bisalkylAAD. A number of studies were initiated to better characterize the differences between these two agents.

The experiment shown in figure 2 was designed to compare the effects of route and schedule of administration on the activities of bisalkylAAD and dihydroxybisalkylAAD against ip P388 leukemia. Schedules and routes of administration were Day 1 only (ip versus iv); Days 1, 5, and 9 (ip versus iv); and Days 1–9 (ip versus sc). Damage to tail veins due to repeated treatment precluded iv drug administration on the daily schedule. Two distinct differences between the two analogs, in addition to the aforementioned tenfold difference in effective dose range, are evident from this study. First, on ip drug administration, dihydroxybisalkylAAD was highly effective on all three treatment schedules (qd, > 309% ILS; q4days, 223% ILS; and one dose, > 309% ILS), whereas bisalkylAAD was less active on a single-dose schedule than on the other two

TABLE 6.—Cross-resistance of anthracycline-resistant sublines of P388 leukemia to aminoanthraquinone derivatives

Compound*	% ILS (long-term survivors/No. treated) at optimal dose†		
	P388‡	P388/adriamycin‡	P388/daunorubicin‡
Adriamycin	88 (1/8) at 6	3 (0/8) at 6	7 (0/8) at 3.6
Mitomycin C	62 (0/8) at 3.6	117 (2/8) at 3.6	93 (0/8) at 3.6
[1]	81 (1/8) at 21.6	17 (0/8) at 13	7 (0/8) at 21.6
[2]	100 (1/8) at 1.3	40 (0/8) at 3.6	36 (0/8) at 2.16
[4]	31 (0/8) at 13	0 (0/8) at 21.6	7 (0/8) at 13
[5]	54 (0/8) at 13	0 (0/8) at 13	0 (0/8) at 13
[6]	38 (0/8) at 13	3 (0/8) at 13	0 (0/8) at 13

*See fig 1.

†Groups of 8 $CD2F_1$ mice were implanted ip with 10^6 P388, P388/adriamycin, or P388/daunorubicin cells on Day 0. Treatment was ip on Days 1, 5, and 9 at 4 logarithmically spaced dose levels of drugs. Long-term survivors 45 days after tumor implantation. Optimal dose is in mg/kg/injection.

‡Median survival times of untreated controls (40 per tumor subline) were 13, 15, and 14 days for P388, P388/adriamycin, and P388/daunorubicin respectively.

schedules (qd, 195% ILS; q4days, 295% ILS; and one dose, 118% ILS). This result was confirmed in another experiment in the ip P388 system with optimal responses for dihydroxybisalkylAAD of > 309% ILS for qd treatment, > 309% ILS for q4days treatment, and > 309% ILS for one-dose treatment, and optimal responses for bisalkylAAD (data not shown) of > 309% ILS for qd treatment, > 309% ILS for q4days treatment, and 114% ILS for one-dose treatment. This result was also observed in the ip L1210 leukemia (data not shown). Second, on the intermittent and single-dose schedules, dihydroxybisalkylAAD had equivalent activity when administered either iv or ip, whereas bisalkylAAD had very little activity when not administered directly in the site of tumor implantation. On the daily treatment schedule, both drugs were considerably more effective when given ip than when given sc.

The clear-cut advantage of dihydroxybisalkylAAD over bisalkylAAD when administered at a site other than that of tumor implantation was investigated further by comparing the effects of the two analogs against systemic (iv implanted) P388 and L1210 leukemias (fig 3). Both drugs were clearly less effective against systemic leukemia than against ip implanted tumors; long-term survivors were not produced in animals inoculated iv with leukemia cells. In iv P388 leukemia (fig 3A) dihydroxybisalkylAAD administered ip on the daily, intermittent, or single-dose schedule resulted in a similar degree of activity at the optimal dose (qd, 96% ILS; q4days, 64% ILS; and one dose, 87% ILS). BisalkylAAD, in contrast, was virtually inactive, with the greatest ILS being 18% at 18 mg/kg administered on Days 1–9. In iv L1210 leukemia (fig 3B), unequivocal superiority for dihydroxybisalkylAAD was also observed (qd, 125% ILS; q4days, 87% ILS; and one dose, 104% ILS as opposed to qd, 51% ILS; q4days, 42% ILS; and one dose, 14% ILS for bisalkylAAD). In another trial against iv P388 leukemia (data not shown) dihydroxybisalkylAAD had a similar degree of activity on three schedules whether administered ip or iv, whereas bisalkylAAD was either marginally active or inactive by either route of administration.

The influence of schedule of administration on the activity of dihydroxybisalkylAAD and bisalkylAAD was also investigated in a solid tumor system, ip colon carcinoma 26 (fig 4). The greater activity observed for dihydroxybisalkylAAD in the two experiments summarized in table 5 was confirmed in this experiment. DihydroxybisalkylAAD was highly active on daily (239% ILS, four of ten 75-day survivors) and intermittent (> 300% ILS, six of ten survivors) treatment, and was somewhat less effective as a single dose (136% ILS, two of ten survivors). BisalkylAAD had respectable activity on daily (100% ILS, two of ten survivors) and intermittent (89% ILS) treatment, but had only marginal activity (36% ILS) as a single dose.

BisalkylAAD and dihydroxybisalkylAAD were compared in a panel of sc implanted solid tumors with respect to effects on tumor growth and survival time (table 7). Unlike their high degree of activity in the leukemias and ip implanted solid tumors described above, these drugs had minimal effects on the sc solid tumors. In general, only 40%–70% inhibition of tumor growth could be achieved at maximally tolerated doses of either analog and no prolongation of survival was obtained. No differences in the activity of the analogs were evident.

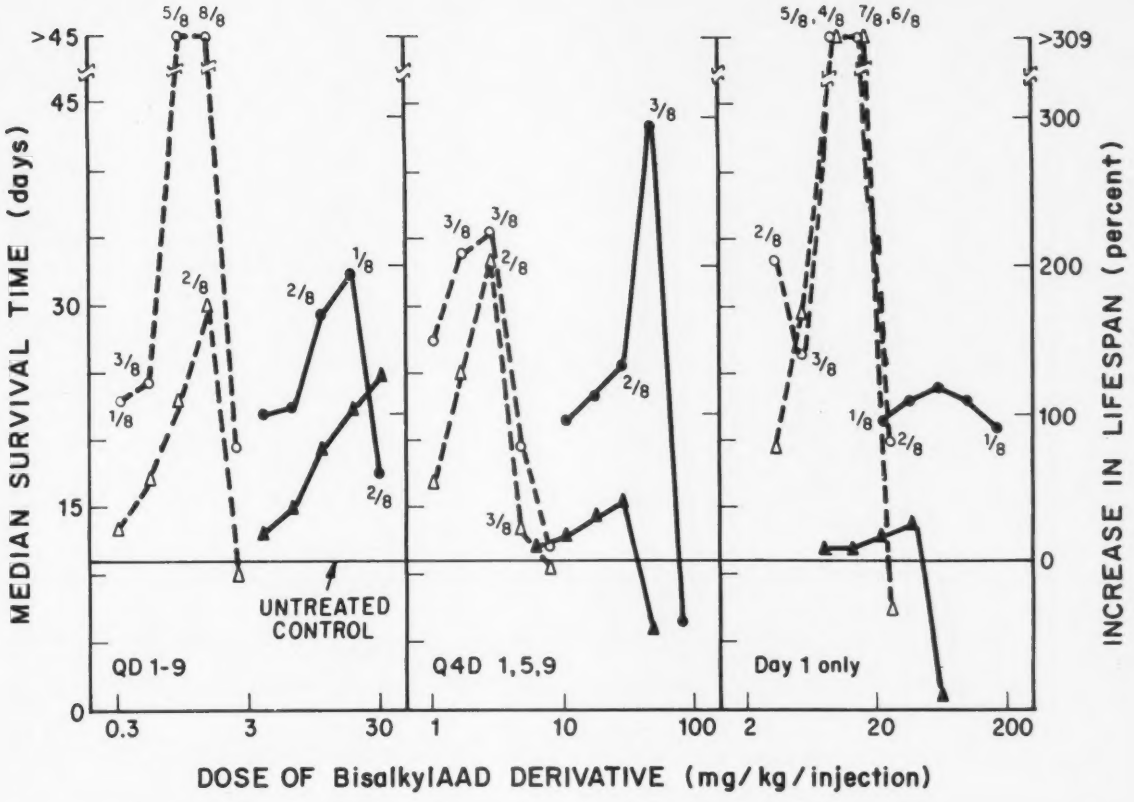


FIGURE 2.—Comparison of bisalkylAAD [*1*] and dihydroxybisalkylAAD [*2*] against ip P388 leukemia. Each point represents median survival time of 8 $CD2F_1$ mice implanted ip with 10^6 P388 leukemia cells (48 mice in untreated control group). BisalkylAAD is represented by the solid lines and closed symbols. DihydroxybisalkylAAD is represented by broken lines and open symbols. Circles represent ip drug administration. Triangles represent iv drug administration on the q4days, Days 1, 5, and 9 and the Day-1 only schedules and sc drug administration on the qd, Days 1–9 schedule. Fractions are tumor-free survivors on Day 45.

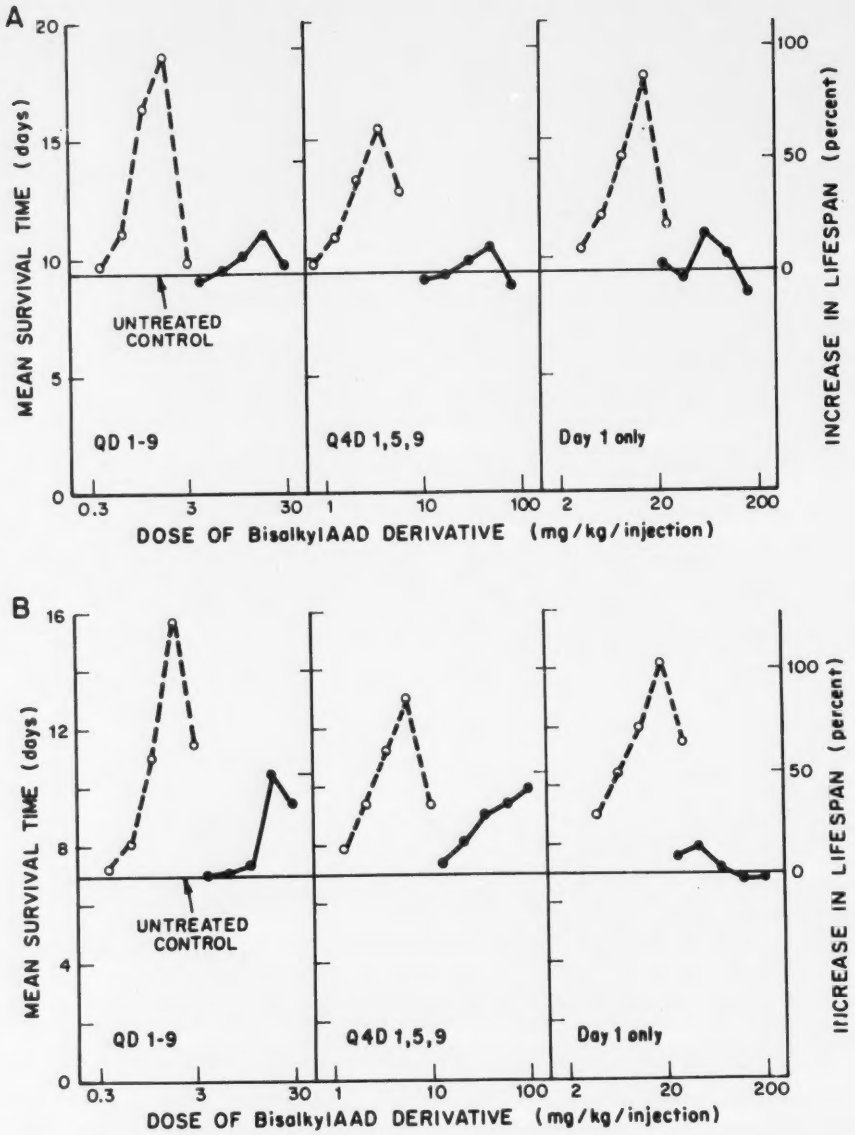


FIGURE 3.—Comparison of bisalkylAAD [*1*] and dihydroxybisalkylAAD [*2*] against iv P388 leukemia (panel A) and iv L1210 leukemia (panel B). Each point represents mean survival time of 8 $CD2F_1$ mice implanted iv with 10^6 P388 or 10^5 L1210 leukemia cells (32 mice in untreated control group). Drugs were administered ip on the schedules indicated. BisalkylAAD is represented by solid lines and closed circles. DihydroxybisalkylAAD is represented by broken lines and open circles.

TABLE 7.—Comparison of bisalkylAAD [*1*] and dihydroxybisalkylAAD [2] against a spectrum of sc implanted solid tumors in mice*

Tumor system	% tumor growth inhibition (% ILS) at optimal dose	
	BisalkylAAD [*1*]	DihydroxybisalkylAAD [2]
Colon carcinoma 38, sc	68 (15) at 40 mg/kg	77 (15) at 4 mg/kg
M5076 ovarian carcinoma, sc	58 (0) at 40 mg/kg	36 (0) at 1 mg/kg
B16 melanoma, sc	60 (0) at 20 mg/kg	70 (0) at 2 mg/kg
Lewis lung carcinoma, sc	58 (0) at 40 mg/kg	44 (13) at 4 mg/kg
Glioma 26, sc	53 (0) at 40 mg/kg	72 (0) at 1 mg/kg

*Compounds were administered ip on Days 1, 5, 9, 13, and 17 to groups of 10 B6D2F$_1$ mice (20 mice in untreated control group). BisalkylAAD was administered at 80, 40, 20, and 10 mg/kg. DihydroxybisalkylAAD was administered at 8, 4, 2, and 1 mg/kg. Tumors were measured twice per week and tumor growth inhibition was determined on the day when the average volume of untreated control tumors surpassed 1000 mm^3.

Comparison of Peracute and Delayed Toxic Effects of BisalkylAAD [*1*] and DihydroxybisalkylAAD [2] in Mice

In the course of therapeutic studies, it was noted that single doses of bisalkylAAD that were therapeutically effective when administered ip were immediately lethal when administered iv (*see* fig 2). Studies were undertaken in non-tumored mice to quantitate the toxic effects of bisalkylAAD and to determine whether this peracute lethal side effect of iv administration was also a property of dihydroxybisalkylAAD.

The two analogs were administered iv, ip, or sc to groups of ten female and ten male mice over a wide range of closely spaced doses. The results for male mice are shown in figure 5. The findings in female mice were similar, except that females were slightly more sensitive to the peracute and delayed toxic effects of both drugs than were males. Iv administration of the diacetate salt of bisalkylAAD (NSC-287513) at doses ≥ 80 mg/kg (145 μmols/kg) resulted in immediate convulsions lasting about 10 seconds, which were followed by prostration and respiratory distress with intermittent periods of apnea. Mice recovering from this acute toxicity resumed normal behavior and appeared completely normal by 45 seconds. Doses of bisalkylAAD that produced delayed toxicity could not be achieved by the iv route of administration. Delayed toxicity following the administration of high doses of bisalkylAAD by the ip or sc route was characterized by progressive cachexia, diarrhea, and death between 3 and 7 days after drug administration. Even extremely high doses of bisalkylAAD (645 mg/kg) given ip or sc did not result in convulsions and immediate death. Thus, with iv administration the dose of bisalkylAAD was limited by the peracute toxicity, and the margin of safety for iv administration, defined as the ratio of the LD10 for acute toxicity to the LD10 for delayed toxicity (which is equivalent to the optimal therapeutic dose), was 0.46.

The acute iv toxicity of dihydroxybisalkylAAD, which is clearly more potent than bisalkylAAD with respect to delayed toxicity (and thus to therapeutic dose range), was quantitatively and qualitatively different from that of bisalkylAAD (fig 5B). DihydroxybisalkylAAD was less potent than the parent compound in producing acute convulsions and death. Doses of ≥ 142 mg/kg (324 μmols/kg) were required to produce peracute lethality. At acutely lethal dose levels of dihydroxybisalkylAAD, convulsions did not start until 30 seconds after drug administration. Convulsions lasted about 40 seconds before the onset of prostration, respiratory distress, and subsequent respiratory arrest. As with bisalkylAAD, mice that recovered from acute toxicity did so within 1 or 2 minutes and showed no further evidence of ill effects. The delayed toxicity of dihydroxybisalkylAAD was similar to that of the parent compound, although it occurred at much lower dose levels and was evident in mice that survived acute toxicity following iv administration. The margin of safety for iv administration of dihydroxybisalkylAAD was 7.9.

These experiments were repeated using both the free bases (formulated as dihydrochlorides) and diacetate salts of bisalkylAAD and dihydroxybisalkylAAD. The salt form had no influence on the acute or delayed toxic dose levels when dose levels were expressed on a molar basis to account for the higher molecular weight of the diacetate salts. Nontoxic dose levels of both compounds administered sc resulted in local toxicity with sloughing of the skin indicative of potential extravasation toxicity.

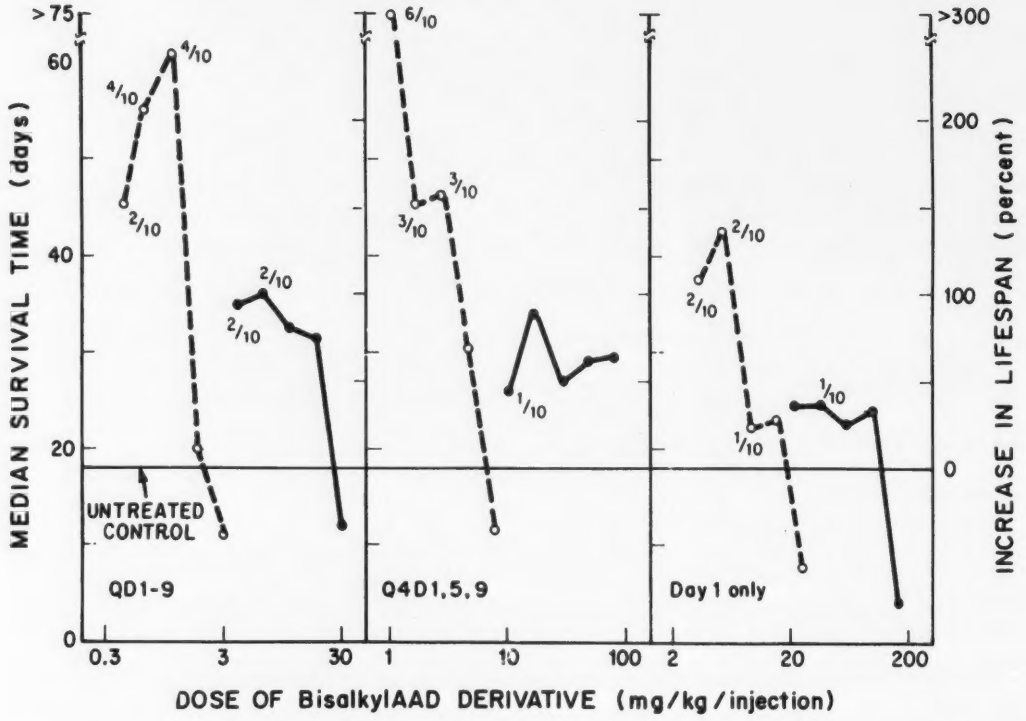


FIGURE 4.—Comparison of bisalkylAAD [1] and dihydroxybisalkylAAD [2] against ip colon carcinoma 26. Each point represents the median survival time of 10 $CD2F_1$ mice implanted ip with 0.5 ml of a 1% tumor brei (50 mice in untreated control group). Drugs were administered ip on the schedules indicated. BisalkylAAD is represented by solid lines and closed circles. DihydroxybisalkylAAD is represented by broken lines and open circles. Fractions are tumor-free survivors on Day 75.

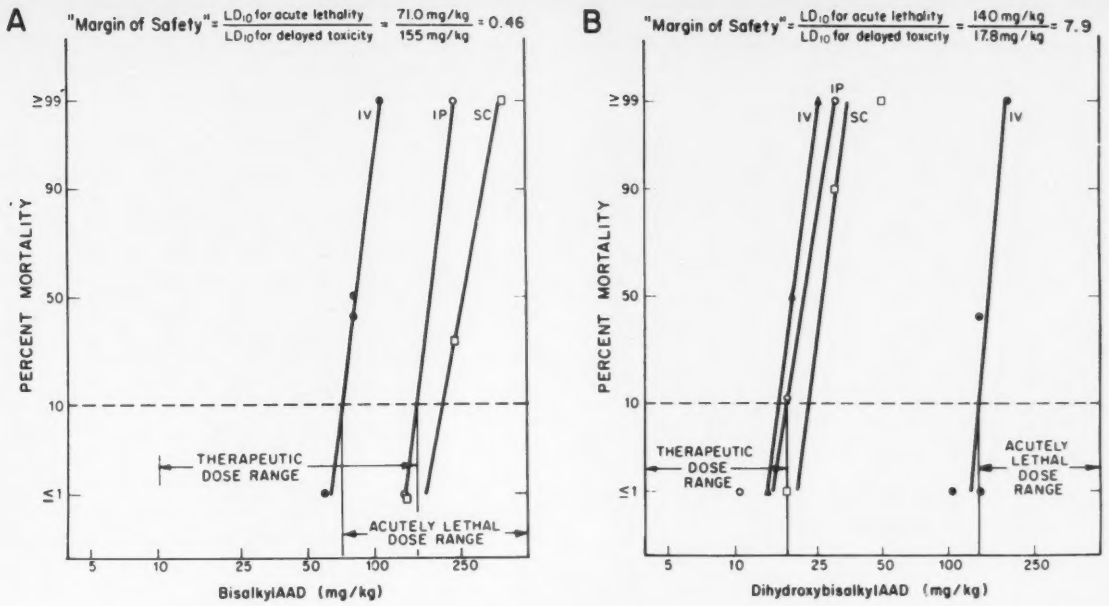


FIGURE 5.—Acute and delayed toxicity of bisalkylAAD [*1*] (panel A) and dihydroxybisalkylAAD [*2*] (panel B) to mice following administration of single doses via several routes of administration. Groups of 10 $CD2F_1$ male mice were treated ip, sc, or iv with a single dose of the drugs at 10 closely spaced dose levels. Acute toxicity represents death within 1 hr and mice were held for 21 days to determine delayed toxicity. The LD10 for delayed toxicity is about equivalent to the optimal therapeutic single dose. Thus, the ratios of the acute and delayed LD10 doses represent a margin of safety from acute toxicity on iv administration. In panel B closed circles represent acute toxicity following iv administration and closed triangles represent delayed toxicity following iv administration. Delayed toxicity following iv administration of bisalkylAAD could not be assessed. Curves were fitted and LD10 values were estimated by a modification of Karber's method (9).

Binding of Aminoanthraquinone Derivatives to DNA and Inhibition of Nucleic Acid Synthesis In Vitro

Fourteen of the aminoanthraquinones evaluated for antitumor activity were studied in vitro for their ability to bind to DNA, as measured by stabilization of double helical DNA to thermal denaturation, and for effects on DNA and RNA synthesis in cultured L1210 cells (table 8). This study included several derivatives which had no antitumor activity in vivo as well as the active aminoanthraquinones. The known DNA intercalators, adriamycin, daunorubicin, actinomycin D, and ellipticine, were evaluated on the same protocols and are included in table 8 for comparison. All of the aminoanthraquinone derivatives tested appeared to bind to DNA and inhibit nucleic acid synthesis. Most compounds gave evidence of being strong DNA binding agents, with ΔTm values (ΔTm = upward shift in DNA melting temperature) as great as or greater than those produced by the anthracycline antibiotics. The derivatives with one basic side chain, [*3*] and [*20*], produced smaller shifts in ΔTm than the rest of the compounds, which have two basic side chains. All compounds caused 50% inhibition of uridine and/or thymidine incorporation by cultured L1210 cells at concentrations < 5 μM. There did not appear to be preferential inhibition of either DNA or RNA synthesis with any of the aminoanthraquinones such as was observed with actinomycin D. The IC50 (concentration which produces 50% inhibition of precursor incorporation) for RNA synthesis was, for those compounds with the greatest differences between IC50 values, only twofold lower than the IC50 for DNA synthesis.

DISCUSSION

Double and Brown (3) synthesized five diethylaminoisopentylaminoanthraquinone derivatives based on the resemblance of this structure (electron-rich planar ring system with a side chain possessing a basic amino group) to antiprotozoal and antineoplastic DNA intercalating agents. These compounds were shown by spectrophotometric tech-

TABLE 8.—Comparison of known DNA intercalators with aminoanthraquinone derivatives with respect to effects on thermal denaturation of double helical DNA and incorporation of labeled thymidine and uridine by L1210 cells in vitro

Compound*	ΔTm† (°C)	IC50† (μM) Thymidine incorporation	IC50† (μM) Uridine incorporation
Adriamycin	13.6	1.5	0.7
Daunorubicin	11.3	1.0	0.3
Actinomycin D	7.1	0.40	0.009
Ellipticine	3.8	4.0	2.8
[*1*]	12.6	1.9	1.6
[2]	15.9	0.34	0.17
[3]	9.2	3.5	3.5
[*4*]	19.7	1.3	0.93
[5]	13.4	1.8	1.4
[6]	11.0	1.3	1.6
[7]	11.4	2.8	1.4
[*9*]	18.5	0.72	0.81
[*10*]	17.0	5.7	3.8
[*11*]	17.8	2.3	1.1
[*12*]	18.0	0.20	0.16
[*13*]	14.2	3.2	2.5
[*14*]	17.4	1.4	2.3
[*20*]	5.7	6.6	4.1

*See fig 1.

†Values listed are averages of duplicate or triplicate experiments performed as indicated in Materials and Methods section. ΔTm = upward shift in DNA melting temperature. IC50 = concentration which produces 50% inhibition of precursor incorporation.

niques to intercalate DNA and have association constants similar to those of adriamycin and daunorubicin. The alkylaminoethylamino- and alkylaminopropylamino-anthraquinone derivatives that are the subject of this paper also appear to intercalate, as evidenced by stabilization of double helical DNA to thermal denaturation and inhibition of the incorporation of labeled thymidine and uridine into TCA-insoluble material of L1210 cells. Also suggestive of DNA binding is the observed cross-resistance to active aminoanthraquinone derivatives in anthracycline-resistant sublines of P388 leukemia. These resistant lines have been shown to be cross-resistant to a number of structurally dissimilar DNA intercalating agents (6).

It is most important to note, however, that ability to increase the melting temperature of DNA and to inhibit precursor incorporation in vitro does not correlate at all with in vivo antitumor activity. Compounds which are totally devoid of antitumor activity in vivo, notably compounds [*9*] and [*12*], produce dramatic shifts in ΔTm and are more potent with respect to inhibiting nucleic acid synthesis than several active derivatives. The relationship of structure of aminoanthraquinone derivatives to antitumor activity and to in vitro effects is summarized in figure 6. The discordance between in vivo antitumor activity and apparent DNA binding capability raises the question of the importance of some effect other than DNA intercalation which might account for the extensive antitumor activity of some of these aminoanthraquinones.

With respect to the relationship between structure and in vivo antitumor activity, it is apparent that several structural features are necessary for significant antitumor activity.

1. The presence of a group with a hydrogen atom available for hydrogen bonding in the side chain (Y in fig 6) appears to be an absolute requirement for activity as evidenced by the complete absence of activity in compounds [*18*] and [*19*]. This is similar to structural requirements for activity of anthracyclines; anthracycline aglycones and anthracyclines in which the amino sugar has been replaced with a non-nitrogenous moiety are devoid of activity (10). However, in the anthracyclines, abolition of basicity by *N*-acylation has resulted in highly efficacious derivatives, albeit at high dose levels (11).
2. The length of the alkyl group between the two nitrogens in the side chain(s) is critical to activity in vivo. Lengthening this constituent (X in fig 6) from two to three carbon atoms results in abolition of antitumor activity as is evident from comparison of compounds [*1*] with [*10*], [7] with [*11*], or [8] with [*20*].
3. The substituent off of the terminal nitrogen atom in the side chain(s) (Z in fig 6) is also critical with respect to antitumor activity. A hydroxyethyl substituent appears to be optimal for antitumor activity, although compounds [5] and [6] with the smaller methyl or primary amine substituents are also quite active. The reason for the definite inferior activity of the ethyl-substituted compound [*4*] is unclear. There is definite bulk intolerance in this position since the propyl, [*14*], and hydroxyethylaminoethyl, [*17*], derivatives as well as the piperazinyl, [*15*], and piperidinyl, [*16*], analogs have little or no activity. The lack of activity of the latter two compounds is not due to the presence of a tertiary rather than a secondary nitrogen since the dimethyl derivatives, [7] and [8], have antitumor activity.
4. The presence of two basic side chains is not required for antitumor activity since compounds [3] and [8], which have a hydroxyl group in place of the alkylaminoethylamino side chains in the 4 position, have activity similar to that of their respective double side chain analogs, [*1*] and [7].

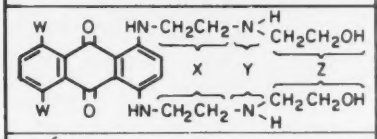
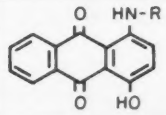
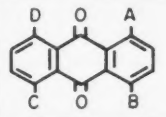
Substitution	Compound	Antitumor Activity	Evidence of DNA Binding
			
W: -H	1	+++	yes
W: -OH	2	+++	yes
X: $-CH_2CH_2-$	1	+++	yes
X: $-CH_2CH_2CH_2-$	10	—	yes
Y: $-N<$	1	+++	yes
Y: $-CH_2-$	18	—	n.t.
Y: -S-	19	—	n.t.
Z: -H, -H	6	++	yes
Z: -H, $-CH_3$	5	++	yes
Z: -H, $-CH_2CH_3$	4	+	yes
Z: -H, $-CH_2CH_2OH$	1	+++	yes
Z: -H, $-CH_2CH_2CH_3$	14	—	yes
Z: -H, $-(CH_2)_2NH(CH_2)_2OH$	17	—	n.t.
Z: $-CH_3$, $-CH_3$	7	+	yes
Z:	15	—	n.t
Z:	16	—	n.t.
			
R: $-(CH_2)_2NH(CH_2)_2OH$	3	+++	yes
R: $-(CH_2)_2N(CH_3)_2$	8	+	n.t.
R: $-(CH_2)_3N(CH_3)_2$	20	—	yes
			
A = B = $-NH(CH_2)_2N(CH_3)_2$	7	+	yes
A = C = $-NH(CH_2)_2N(CH_3)_2$	9	—	yes
A = B = $-NH(CH_2)_3N(CH_3)_2$	11	—	yes
A = C = $-NH(CH_2)_3N(CH_3)_2$	12	—	yes
A = D = $-NH(CH_2)_3N(CH_3)_2$	13	—	yes

FIGURE 6.—Structure-activity relationships among aminoanthraquinone derivatives. Antitumor activity is summarized as follows: +++ = curative to > 40% of mice bearing ip P388, L1210, B16, or colon 26; ++ = > 75% ILS with some cures in mice bearing aforementioned tumors; + = about 50% ILS; − = inactive or only marginally active in ≥ 1 tumor system. Evidence for binding to DNA is based on ability to stabilize DNA to thermal denaturation and inhibit thymidine and uridine incorporation in whole cells. n.t. = not tested.

5. Although the optimal analogs have not been evaluated, it would appear that substitution with alkylaminoalkylamino side chains in position 1 and 4 is optimal as evidenced by comparison of compounds [7] and [9]. The 1,4- 1,5-, and 1,8-bis(dimethylaminopropylamino)-substituted compounds, [11], [12], and [13], are all inactive even though they apparently bind to DNA quite strongly and are potent inhibitors of DNA and RNA synthesis in vitro.
6. Substitution of the ring system distal to the basic side chain(s) has not been adequately evaluated. The dihydroxy substitution in compound [2] increases the electron density of the quinoid ring system and results in a more potent compound with extensive activity, as will be discussed in greater detail below.

It should be noted that structure-antitumor activity relationships among these aminoanthraquinones are consistent in the three tumor systems (ip P388, ip L1210, and ip B16), in which active as well as inactive analogs have been evaluated. It is also interesting that analogs (eg, compounds [9]–[17]) without antitumor activity are toxic to mice at dose levels similar to or only twofold to fourfold higher than dose levels of active analogs; in many classes of antitumor agents, loss of therapeutic activity is paralleled by loss of host toxicity. This observation could suggest that toxicity is due to binding to DNA as is observed with inactive analogs, whereas antitumor activity could be due to some other mechanism.

Driscoll et al (12) reviewed the antitumor screening data of anthraquinone derivatives in the NCI drug development program through 1971. None of 379 anthraquinones tested had significant in vivo activity in the tumor systems evaluated. Several of these anthraquinones had basic side chains and a few compounds (notably compounds designated EN-873 and EN-1039 in that paper) were quite closely related to active structures described herein. The low yield of active compounds among anthraquinone derivatives highlights the rigorous structural requirements for antitumor activity in this class of compounds. It would be most valuable to obtain a number of other derivatives related to the active aminoanthraquinones to more accurately define structure-activity relationships and to develop the best compound(s) for clinical evaluation. For instance, it would be useful to determine whether lengthening the alkyl side chain (X in fig 7) to four or more carbons, as in the compounds described by Double and Brown (3), would provide active antitumor agents. In addition, further investigation of substitution of the ring system distal to the alkylaminoalkylamino side chains should prove fruitful as would synthesis of additional compounds with only one basic side chain. The relative simplicity of synthesis and high yields (*see* ref 1 and table 1) make this an attractive field for further synthesis.

Of the compounds currently available, dihydroxybisalkylAAD is clearly the best candidate for development toward clinical trial. Compound [3] which has a single 2-[2-(hydroxyethyl)amino]ethylamino side chain in the 1 position and a hydroxyl group at position 4 has not been as extensively tested but shows promising activity. Compound [3] is currently undergoing intensive comparison with compound [2].

The superiority of dihydroxybisalkylAAD, [2], over bisalkylAAD, [1], is evident in several characteristics pertinent to activity and toxicity. All of these aminoanthraquinones are extremely strong dark blue dyes. The iv or ip administration of these compounds to mice results in persistent deep blue coloration of internal organs and skin. This potential cosmetic side effect could prove annoying in the clinical use of these agents. The high potency of dihydroxybisalkylAAD relative to the other active agents would result in administration of lower doses with less potential for staining patients blue.

A more serious potential side effect is the immediate death induced by iv administration of doses of bisalkylAAD that are quite close to or overlap the therapeutic dose range on intermittent or single-dose regimens. The immediate and transient nature of this effect, the behavior of intoxicated mice, and the absence of the effect on ip or sc drug administration suggest a bolus effect of high concentrations of bisalkylAAD on cardiac or nervous system function. Indeed, studies of bisalkylAAD in dogs have shown that the drug causes acute cardiovascular toxicity characterized by decreased cardiac output, increased pulmonary and vascular resistance, and elevated blood pressure (Guarino AM, NCI, personal communication). Although this toxicity might prove to be avoidable by slow iv infusion, it still could hinder clinical use of this drug. The increased potency of dihydroxybisalkylAAD with respect to therapeutic dose range is not paralleled by increased potency with regard to induction of convulsions and respiratory arrest on iv administration. Thus, this toxicity would be unlikely to prove a hindrance to the use of this analog. The active compounds of this series were not active orally (unpublished screening results of the NCI) and had local toxicity upon sc administration, so it is likely that iv administration is required. The acute iv toxicity of the other active and inactive analogs in figure 6 has not been studied extensively. We have, however, observed in preliminary studies that single iv doses of 40–80 mg/kg of all of the active and

several of the inactive derivatives induce convulsions and/or death within 5 minutes of administration to mice.

Apart from differences in toxicity, dihydroxybisalkylAAD appears to be superior to bisalkylAAD with respect to therapeutic activity as well. Both compounds have curative activity against ip P388 leukemia and ip B16 melanoma and both have, at best, marginal activity against several sc implanted solid tumors. These tumor systems do not differentiate between the two analogs. DihydroxybisalkylAAD has shown reproducibly better activity than bisalkylAAD in several other tumor systems: ip L1210 leukemia, ip colon carcinoma 26, anthracycline-resistant sublines of P388 leukemia, and iv P388 and L1210 leukemias. The aminoanthraquinones are not devoid of activity in sc implanted solid tumors. Results obtained in another laboratory (Corbett TH, Southern Research Institute, personal communication) have shown bisalkylAAD and dihydroxybisalkylAAD to be quite active against sc mammary adenocarcinoma 16C and colon carcinoma 06A. It was also observed that bisalkylAAD administered iv (acute toxicity necessitating a low-dose, intermittent treatment schedule) was highly active against sc colon carcinoma 38, in contrast to our results with ip drug administration. DihydroxybisalkylAAD was found to be significantly less active than bisalkylAAD in this setting.

The greater activity of dihydroxybisalkylAAD against systemic leukemias when administered iv suggests that dihydroxybisalkylAAD has more favorable pharmacokinetic characteristics than bisalkylAAD. Cytotoxic concentrations of dihydroxybisalkylAAD can be achieved in tumor cells distant from the site of drug administration to a greater extent than is possible with bisalkylAAD. The observed differences in schedule dependency, mainly the poor activity of single-dose bisalkylAAD relative to repeated treatment, in contrast to results obtained with dihydroxybisalkylAAD, also suggest differences in the pharmacologic characteristics of these two analogs.

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Combination Chemotherapy With Cyclophosphamide, Vincristine, and Prednisone in the Treatment of Refractory Chronic Lymphocytic Leukemia [1]

Martin M. Oken* and Manuel E. Kaplan [2]

SUMMARY

Over a 5-year period, cyclophosphamide, vincristine, and prednisone (CVP) therapy has been studied in 18 patients with advanced refractory chronic lymphocytic leukemia (CLL). Chemotherapy was administered at 3–4-week intervals using either iv or oral cyclophosphamide. Responses were evaluated using criteria based on improvement in peripheral blood lymphocytosis, lymphadenopathy, hepatosplenomegaly, hemoglobin levels, platelet counts, and systemic symptoms. Sixty-seven percent of the patients showed an objective clinical response, while 44% of the total group obtained a clinical complete response or a partial response. The median survival from the diagnosis of CLL was 62 months for the entire group, while the median survival from the start of CVP was 30 months. Patients achieving a clinical complete response or a partial response had a median survival of 37.5 months, while nonresponding patients survived 5 months. In all responding patients, improvement was evident during the first month of therapy. Eight patients had prolonged disease control (1–4 years' duration) either without maintenance therapy or with CVP maintenance therapy. Despite aggressive treatment in a group of patients of whom 89% exhibited pretreatment bone marrow failure, only two treatment deaths were seen, both due to infection. Otherwise, toxicity was generally mild and reversible. Of 11 thrombocytopenic patients, only four had a further decrease in their platelet count and none had serious bleeding. Seven of 12 patients with marked splenomegaly and cytopenia achieved at least a partial remission. Thus, CVP appears effective without undue risk in patients with refractory CLL and may offer an alternative to splenectomy in selected patients.

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Primary treatment of chronic lymphocytic leukemia (CLL) is generally initiated only when patients develop (*a*) systemic symptoms related to their leukemia, (*b*) painful or rapidly increasing lymphadenopathy, (*c*) bone marrow failure, or (*d*) autoimmune destruction of rbcs or platelets (1). Initial disease control is obtained in 55%–80% of patients using either total-body irradiation or chemotherapy with chlorambucil or chlorambucil plus prednisone (1). Eventually however, most patients become refractory to standard therapy and enter a phase of the disease where alternative therapies, when attempted, usually fail to produce consistent or long-lasting disease control (1). Therapeutic approaches in such patients have included leukapheresis (2,3), mediastinal irradiation (4,5), extracorporeal irradiation of blood (6), and, in hypersplenic patients, splenectomy (7) or splenic irradiation (8). Recent reports in which CLL patients were treated with a five-drug regimen (vincristine, BCNU, cyclophosphamide, melphalan, and prednisone) (9) or a three-drug regimen (cyclophosphamide, vincristine, and prednisone; CVP) (10) suggest a role for combination chemotherapy in CLL. Although some of the pa-

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tients in the latter series had received prior single-agent therapy, a systematic analysis of the effectiveness of CVP in refractory CLL has not been reported.

In the present study, the effectiveness of combination chemotherapy with CVP is evaluated in a group of patients with advanced refractory CLL and, in most instances, evidence of bone marrow failure. The results reveal CVP to be an effective, well-tolerated treatment in these high-risk patients.

MATERIALS AND METHODS

From 1972 to 1976, 18 patients with CLL were entered in this study. For this study, the diagnosis of CLL required peripheral blood lymphocytosis (> 15,000 cells/μl) not attributable to a disease other than CLL and a hypercellular bone marrow with lymphocytes comprising ≥ 50% of the nucleated cells at the time of diagnosis. Patients with nodular lymphoma or with diffuse lymphoma associated with focal bone marrow involvement were excluded.

All patients had received prior alkylating-agent therapy either alone or in combination with corticosteroids. In every instance but one, prior chemotherapy consisted of daily oral administration of an alkylating agent, rather than high-dose intermittent therapy. Thirteen of the 18 patients studied had responded one or more times to prior therapy but 17 of the 18 patients were clearly refractory at the time of entry in the study. This included 13 patients who had increasing objective manifestations of disease while receiving alkylating agents at maximally tolerated daily doses for ≥ 3 weeks, or who had no evidence of disease regression after similar treatment for ≥ 4 weeks. A transient increase in lymphocytosis during the first 2–3 weeks of corticosteroid therapy was not considered increasing disease. Four additional patients were considered refractory after demonstrated bone marrow intolerance manifested by marked deterioration of granulocyte and platelet counts during chemotherapy precluded further treatment with daily alkylating agents at adequate doses. The one nonrefractory patient was included because he had massive lymphadenopathy and splenomegaly with demonstrated hypersplenism as well as thrombocytopenia and anemia requiring transfusion and was judged a poor risk for conventional daily oral alkylating-agent therapy. Clinical characteristics of the 18 patients studied are shown in table 1. The staging criteria described by Rai et al (11) are used. In this system, stage II requires hepatomegaly or splenomegaly, stage III requires anemia (hemoglobin level of < 11 g/dl), and stage IV requires thrombocytopenia (platelet count of < 100,000/μl).

In the present study two CVP regimens were used. Cyclophosphamide was administered either as an 800-mg/m^2 iv infusion on Day 1 or as a 400-mg/m^2/day oral dose on Days 1–5. All patients received vincristine (2 mg iv, Day 1) and prednisone (60–100 mg/m^2 orally, Days 1–5). Cycles were repeated at 3–4-week intervals. Because the results obtained with the oral regimen were essentially identical to the results obtained with the schedule using iv cyclophosphamide, they are combined in the analysis of this study. Therapy was initiated at full dose in 12 of the 18 patients. In all patients the dose level in subsequent cycles was adjusted depending on observed myelotoxicity.

Response criteria were based upon changes in four major CLL disease manifestations: (*a*) peripheral blood lymphocytosis; (*b*) palpable disease including lymphadenopathy, hepatomegaly, and splenomegaly; (*c*) bone marrow failure as reflected by anemia or thrombocytopenia; and (*d*) subjective symptoms, including weakness, weight loss, and fever not attributable to infection. Therapeutic responses were graded according to the following criteria: *clinical complete response (CR)*—the complete disappearance of all peripheral evidence of CLL, with no abnormality in categories *a*, *b*, *c*, or *d* (*see above*), a peripheral blood granulocyte count of ≥ 1500/μl, and a peripheral blood lymphocyte count of ≤ 3000/μl; *partial response (PR)*—a ≥ 75% decrease in peripheral blood lymphocyte count, a ≥ 50% improvement in palpable disease, a hemoglobin level of ≥ 11 g/dl, a platelet count of ≥ 100,000/μl (or improvement in either value by ≥ 75% of their deviations from normal), and resolution of all subjective symptoms due to CLL; *improvement*—definite improvement in two or more categories (*a*, *b*, *c* or *d*), but without sufficient resolution of disease to meet PR criteria; and *no response (NR)*—disease progression or no improvement. All patients who received Day-1 therapy are included in the analysis.

Chemotherapy toxicity was graded according to the Eastern Cooperative Oncology Group criteria based on a scale of 0–5: 0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = life-threatening, and 5 = lethal. An abridged version of these criteria is presented in table 2. When the hemoglobin level, platelet count, or neutrophil count did not decrease from pre-CVP baseline levels, this was considered no toxicity even when these values remained low. However, when infection, bleeding, or increased transfusion requirement developed during the time the patient was undergoing CVP therapy, this was analyzed as CVP-related toxicity since it was rarely possible to demonstrate that such complications were entirely the result of disease and not of therapy.

TABLE 1.—Patient characteristics and results

Patient No.	Age/ sex	Prior therapy*	Time from diagnosis to CVP (mos)	Status at start of CVP: Clinical stage	Refractory	Blood lymphocyte count (× $10^3/\mu l$)	Hemoglobin level (g/dl)	Platelet count (× $10^3/\mu l$)	Response	Duration of response (mos)	Duration of disease control (mos)	Survival (mos): From diagnosis	Survival (mos): From start of CVP
1	58/M	P × 2 Clb + P × 1 Cy + P × 3	160	IV	Yes	34.4	7.5	23	Clinical CR	14	28	216	56
2	49/M	UM × 3	103	IV	No	101.7	8.3	48	Clinical CR	29	36	155+	53+
3	58/M	Clb × 2	15	IV	Yes	22.5	10.8	45	PR	12	12	32+	19+
4	77/M	Clb × 1 Clb + P × 4	58	III	Yes	81.4	10.1	141	PR	22	39	98	40
5	56/M	Cy × 1 Clb × 1 Cy + P × 1 Clb + P × 1 P × 1	36	IV	Yes	115.0	12.1	78	PR	7	10	49	13
6	63/M	Clb × 1	27	III	Yes	155.0	11.6	156	PR	11	13	62	35
7	76/M	Clb × 3	70	III	Yes	278.2	9.5	140	PR	30	46	124	54
8	52/M	Clb × 3	20	IV	Yes	240.0	8.3	90	PR	6	24+	50+	30+
9	66/M	Clb × 1 Clb + P × 5	44	IV	Yes	22.0	10.8	35	Improvement		1	52	8
10	62/M	Cy + P × 3 Cy × 1 Clb × 1	27	IV	Yes	9.2	13.2	92	Improvement		6	67	40
11	67/F	Clb + P × 2	224	IV	Yes	88.0	9.3	16	Improvement		0	138+	39+
12	66/M	Clb × 3	123	II	Yes	111.0	13.2	117	Improvement		12	153	30
13	61/M	Sp Clb + P × 1	18	III	Yes	200.0	10.2	197	NR			21	3
14	63/M	Clb + P × 1 Clb × 1	6	III	Yes	105.0	6.9	120	NR			11	5
15	67/M	P × 2 Clb + P × 2	22	IV	Yes	32.0	12.3	22	NR			78+	56+
16	58/M	Clb × 1	3	II	Yes	310.0	12.0	176	NR			17	14
17	64/M	Clb × 1 Cy × 1 Cy + P × 1	39	IV	Yes	36.0	9.3	13	NR			41	2
18	50/M	Clb × 1 Clb + P × 1	29	IV	Yes	219.0	6.4	28	NR			34	5

*P = prednisone; Clb = chlorambucil; Cy = cyclophosphamide; UM = uracil mustard; Sp = splenectomy.

RESULTS

All 18 patients treated with CVP were evaluable for response and toxicity. Responding patients included two clinical CRs (11%), six PRs (33%), and four improvements (22%) (tables 1 and 3). Overall, two thirds of all patients responded, 44% obtaining clinical CR or PR. Clinical features correlating with response are listed in table 3. Five of seven patients < 60 years of age achieved clinical CR or PR (71%) compared with only three of 11 patients > 60 years. Five of 11 stage IV patients and three of five stage III patients had clinical CR or PR. The prognostic importance of responsiveness to prior therapy is demonstrated by the observation that, of the five patients who had been refractory to all previously attempted therapy, only one responded to CVP. Hypersplenism was diagnosed in 12 patients who

TABLE 2.—Toxicity criteria

	Eastern Cooperative Oncology Group grade				
	0	1	2	3	4
Hemoglobin level (g/dl)	≥ 11	9.5–10.9	< 9.5	Transfusions required	
Neutrophil count (× $10^3/\mu l$)	≥ 1.9	1.5–< 1.9	1.0–< 1.5	0.5–< 1.0	< 0.5
Platelet count (× $10^3/\mu l$)	≥ 130	90–< 130	50–< 90	25–< 50	< 25
Infection	None	Mild	Requires active treatment	Debilitating	Life-threatening
Hemorrhage	None	Minimal	Moderate, not debilitating	Debilitating	Life-threatening
Hematuria	None	Microscopic	Gross	Gross + clots	Gross with obstruction
Nausea and vomiting	None	Anorexia or nausea	Vomiting	Intractable vomiting	
Neuropathy	None	Decreased deep tendon reflexes; paresthesias, mild; constipation, mild	No deep tendon reflexes; paresthesias, moderate-severe; constipation, moderate-severe; weakness, mild	Severe sensory loss; severe weakness; obstipation; bladder dysfunction	Neuropathy, confining patient to bed or wheelchair

had cytopenia associated with marked splenomegaly and/or accelerated splenic sequestration of ^{51}Cr-labeled rbcs. Seven of these patients achieved clinical CR or PR. Three of the remaining hypersplenic patients had subsequent splenectomy after they had failed to respond to or achieved only transient improvement from CVP. Two had excellent clinical improvement after splenectomy and the third died of postoperative infection.

All eight patients responding with a clinical CR or a PR did so within the first 4 weeks of therapy. Six patients not responding during the initial 4 weeks of therapy received three or more additional cycles of CVP. Only one of these patients obtained useful clinical improvement. No difference in response rate was noted between the ten patients receiving CVP with oral cyclophosphamide and the eight patients receiving the iv cyclophosphamide regimen. The median response duration for the eight clinical CR plus PR patients was 12 months (range, 6–30). Relapse was signaled by the gradual increase in lymphocytosis, lymphadenopathy, and, in some instances, splenomegaly. Despite relapse, disease control was adequate in most patients so that resumption or change in therapy was not required for an average of 1 year after relapse. Thus, eight patients refractory to prior chlorambucil therapy experienced prolonged disease control of 1–4 years' duration. The average period of disease control in clinical CR or PR patients was 24 months with considerably shorter periods of control in the patients showing clinical improvement only.

Survival data are presented in figure 1 and in tables 1 and 2. The median survival for all patients studied was 30 months from initiation of CVP therapy. Patients entering clinical CR, PR, or improvement had a median survival of 37.5 months; nonresponders had a median survival of 5 months. Only two of the nonresponding patients survived > 1 year after initiation of CVP. Age, clinical stage of disease, and presence or absence of clinical hypersplenism did not predict survival in this series.

As expected in a group in which 16 of 18 patients had significant pretreatment bone marrow failure, the toxicity encountered was mainly hematologic (table 4). Two treatment-related deaths occurred. Both were caused by infection associated with profound neutropenia. A third patient developed septic arthritis, but recovered. Of 11 stage IV (thrombocytopenic) patients, only four developed further decreases in platelet count and only one of these patients had purpura. Serious bleeding was not

TABLE 3.—Clinical response to CVP

	No. of—				Median survival in mos from start of CVP (range)
	Patients treated	Clinical CR + PR	Improvements	NR	
All patients	18	8	4	6	30 (2–56)
Stage					
II	2	0	1	1	— (14, 30)
III	5	3	0	2	35 (3–54)
IV	11	5	3	3	30+ (2–56)
Age					
< 60 yrs	7	5	0	2	19+ (5–56)
> 60 yrs	11	3	4	4	35 (2–55+)
Initially responsive to prior therapy	13	7	4	2	39+ (2–56)
Refractory to all prior therapy	5	1	0	4	5 (3–35)
Hypersplenism	12	7	3	2	35 (5–56)
Response (clinical CR, PR, or improvement) not observed by 4 wks	8	0	2	6	9 (2–55+)

encountered. Peripheral neuropathy from vincristine was seen in five patients and consisted of paresthesias, hyporeflexia, and, in two patients, mild but nondisabling weakness. Cyclophosphamide-induced hemorrhagic cystitis proved to be a minor problem and was encountered in only two patients.

DISCUSSION

CVP has been evaluated as a therapy for patients with CLL (10); however, a detailed assessment of its effectiveness in refractory disease has not been previously reported. In a recent study (10), this regimen produced a response rate of 72% in 36 CLL patients of various stages. Thirteen patients in that study had received prior therapy, including nine patients with stage III or IV disease. The number judged to be refractory to prior therapy was not reported. The rationale for the use of CVP in CLL is based on the demonstrated effectiveness of CVP and similar regimens in producing remissions in diffuse lymphocytic non-Hodgkin's lymphomas, both the well-differentiated (D-WDLL) and the poorly differentiated (D-PDLL) types (12–14). The frequent, though not constant, histopathologic similarity between CLL and D-WDLL has been described (15–17). The use of high-dose intermittent alkylating-agent therapy in CLL is further supported by the demonstrated efficacy and modest toxicity of biweekly chlorambucil in CLL (18). High-dose intermittent chlorambucil with prednisone appeared only slightly better than low-dose chlorambucil with prednisone in a recently published randomized study (19). Chlorambucil with prednisone has been demonstrated to be somewhat more effective than chlorambucil (20) or prednisone (19) alone in CLL. However, the specific role of vincristine in the treatment of CLL remains in doubt and is relatively unstudied. While one study showed vincristine to be ineffective in all six CLL patients studied (21), two other studies involving a total of five patients showed evidence of therapeutic activity for vincristine in three of the patients (22,23).

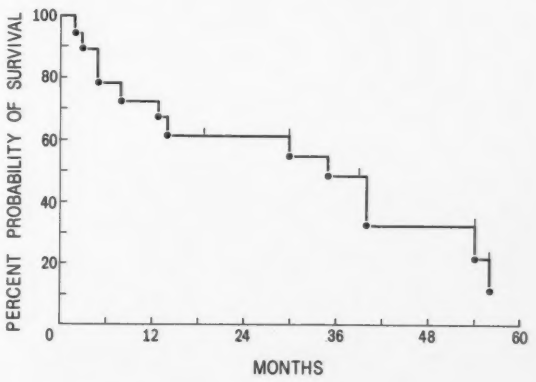

FIGURE 1.—Actuarial survival of CLL patients from start of CVP.

TABLE 4.—Toxicity attributed to CVP

Toxic effect	Degree of toxicity (No. of patients at each grade)			
	0	1–2	3–4	5
Anemia	10	6	2	0
Thrombocytopenia	9	5	4	0
Bleeding	17	1	0	0
Infection	14	1	1	2
Gastrointestinal	16	2	0	0
Neurologic	13	5	0	0
Cystitis	16	2	0	0
Other	17	1	0	0
Most severe toxicity	3	9	4	2
Most severe hematologic toxicity	5	7	4	2

This study demonstrates that CVP is an effective mode of therapy in many patients with advanced refractory CLL. Further studies would be required, however, to define the contribution of vincristine to the overall effectiveness of the regimen. Bone marrow failure, hypersplenism, and age > 60 years did not decrease the chance of response to therapy in this series. However, patients who have previously received alkylating-agent therapy without response appear unlikely to respond to CVP. This requires further study since the number of such patients treated in this series is small.

In order to compare results in the present series with reported results from other centers, our results were analyzed using several of the reported response criteria (table 5). A comparable response rate is obtained using these several criteria.

Toxicity was moderate, particularly in view of the compromised pretreatment bone marrow status of most patients studied. Nevertheless, the incidence of severe toxicity (33%) was somewhat greater than that reported for chlorambucil and prednisone combinations (19). Other than hematologic toxicity, however, no severe toxic effects were observed. Although cystitis, nausea and vomiting, and peripheral neuropathy occurred in a minority of patients, invariably they were mild and reversible. The use of vincristine appeared to add little to the toxicity of the regimen in this group of patients.

The 30-month median survival observed from the start of CVP compares favorably with the median survival previously reported for stage III and IV patients (11). Responsiveness to CVP therapy was associated with a sevenfold superiority in survival in this study. Thus, it appears that this regimen is capable of increasing survival in a group of previously treated refractory patients with long-standing CLL.

In the hypersplenic patients included in this study, the 55% incidence of clinical CR and PR and the 35-month median survival from the start of CVP are of particular interest. This is comparable to reported response rates with splenectomy (24) or splenic irradiation (8) in hypersplenic refractory CLL. Two patients receiving CVP who failed to achieve disease control had good responses to subsequent splenectomy. These results support the use of CVP as an alternative to splenectomy in patients with hypersplenism associated with refractory CLL. In patients failing to respond to CVP within 1 month, splenectomy or splenic irradiation could be considered.

TABLE 5.—Results of present study utilizing various response criteria

Source of response criteria (ref No.)	Response			
	Clinical CR/CR	PR/good	Improvement/ fair	NR
Present series	2	6	4	6
Cancer and Acute Leukemia Group B (18)	1	6	4	7
Southeastern Cancer Study Group (17)	2	7	5	4
Roswell Park Memorial Institute (19)		7	5	6
University of Michigan Medical School (10)	2	5	5	6

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Schedule-Dependency Assessments of Ribonucleoside Diphosphate Reductase Inhibitors When Used in Combination With Platinum Compounds Plus Cyclophosphamide in the Treatment of Advanced L1210 Leukemia [1,2]

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SUMMARY

Each of three ribonucleoside diphosphate reductase inhibitors was used as a third drug in combination with selected antitumor platinum (Pt) agents and cyclophosphamide (CY) in the treatment of advanced L1210 leukemia in C57BL/6 × DBA/2 mice. Each was synergistic with the various Pt plus CY combinations but the effect was highly schedule dependent. The collective cure rate was 68% when hydroxyurea (HU) was given as a single injection with Pt plus CY; the cure rate was 15% when HU was administered on a divided-dose schedule with Pt plus CY. The collective cure rate was 53% when guanazole was given as a single injection with Pt plus CY, but was only 8% when it was given on a divided-dose schedule with Pt plus CY. The effect of 4-methyl-5-amino-1-formylisoquinoline thiosemicarbazone, when used as a third drug with the various Pt plus CY regimens, was not schedule dependent as assessed by the collective cure rate. A therapeutic synergy between CY and each of the three ribonucleoside diphosphate reductase inhibitors was also observed.

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Earlier reports from this laboratory described significant potentiation by hydroxyurea (HU) when combined with cyclophosphamide (CY) and various antineoplastic platinum (Pt) compounds in the treatment of L1210 leukemia (1–3). Those studies, however, were done using a single injection of each drug on Day 1 or 3 following ip implantation of 10^6 L1210 cells. In the most recent of these reports (3), the collective cure rate (> 60-day survival) was 11% when CY was combined with each of seven organoplatinum congeners, and was increased to 53% when a single injection of HU at a dose of 1000 mg/kg was added to the treatment regimen.

The mechanism by which HU so markedly enhances the antileukemic action of Pt plus CY is not clear. HU was first synthesized > 100 years ago. It is a simple analog of urea, but chemically may be considered to be the hydroxamate of carbamic acid. It is rapidly excreted in the urine (4,5) and its principal mechanism of action is the inhibition of ribonucleoside diphosphate reductase (6–9). Rapid urinary excretion and selective inhibition of ribonucleoside diphosphate reductase are properties which HU shares with guanazole (GZ), an aryl diamino-substituted triazole with antineoplastic properties (10–14). The chemically dissimilar 4-methyl-5-amino-1-formylisoquinoline thiosemicarbazone (MAIQ–1), rationally designed and synthesized by Agrawal et al (15), has significant activity against a number of transplantable tumors and inhibits ribonucleoside diphosphate reductase from Novikoff rat tumor at extremely low concentrations ($I50 = 6 \times 10^{-8}$ M).

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However, its biologic half-life is approximately one order of magnitude greater than that of HU or GZ (16).

In spite of the structural differences among HU, GZ, and MAIQ–1, they share the singular property of binding to iron (7,8,16, and unpublished observations). A common mode of antitumor action among the three classes of reductase inhibitors may be inferred from the work of Schabel et al (13), which showed that a variant of the L1210 leukemia selected for resistance to HU was also resistant to GZ and picolinaldehyde thiosemicarbazones; the latter are structural analogs of MAIQ–1 which also inhibit the reductase system. It has been postulated that the iron chelate of MAIQ–1 may be the active tumor-inhibitory form of this compound (16). In contrast, it has been shown that the inhibition of DNA synthesis and of tumor growth induced by HU is actually enhanced by the iron-chelating agent, deferoxamine, and this augmentation is abrogated by an excess of iron (17). An antagonism by ferrous iron of the action of HU on ribonucleoside diphosphate reductase has also been reported (9). Since the B2 protein subunit of the reductase system contains two atoms of non-heme iron per molecule, and HU interacts with this component to yield a modified absorption spectrum (8), it seems possible that the common property of iron binding by HU, GZ, and MAIQ–1 may be related to their basic modes of action at the molecular level.

The work reported here was initiated to determine if GZ and MAIQ–1 are as effective as HU in potentiating the action of CY plus various Pt compounds against advanced (Day 3) L1210 leukemia, as well as to determine if the different rates of excretion of HU and GZ compared with MAIQ–1 influence in any way the schedule dependency of the three-drug regimen.

MATERIALS AND METHODS

The parent compound, *cis*-dichlorodiammineplatinum(II) (*cis*-DDP), was synthesized by the method of Kauffman and Cowan (18). Two of the organoplatinum analogs chosen for this study have undergone limited clinical trials [malonato(1,2-diaminocyclohexane)platinum, NSC-224964; and sulfato(1,2-diaminocyclohexane)platinum, NSC-250427] (19–22), and another may be a candidate for preclinical toxicology tests [4-carboxyphthalato(1,2-diaminocyclohexane)platinum, NSC-271674]. The methods of syntheses of these analogs were reported earlier (23,24). HU was purchased from ICN Pharmaceuticals, Inc, CY was purchased from Mead Johnson Laboratories, and GZ and MAIQ–1 were supplied by the Drug Synthesis and Chemistry Branch, Developmental Therapeutic Program (DTP), Division of Cancer Treatment (DCT), National Cancer Institute (NCI). The L1210 leukemia was propagated by serial passage in DBA/2 mice, and testing was done in (C57BL/6 × DBA/2)F_1 (BDF_1) hybrids; both strains were supplied by the Mammalian Genetics and Animal Production Section, Drug Evaluation Branch, DTP, DCT, NCI.

The experimental protocol was substantially the same as described previously (1–3,25). Twenty groups of ten BDF_1 mice were inoculated ip with 10^6 L1210 cells on Day 0. Six groups of ten mice were used as controls in each experiment. Treatment was via the ip route on Day 3 with CY and Pt, as well as with HU, GZ, or MAIQ–1 when each was being administered as a single injection. Otherwise, each of the reductase inhibitors was administered at the same total dose but was divided into four individual injections which were administered on Days 3–6. Doses of each Pt compound and of CY which would yield only moderate increases in survival times were chosen. The dose of HU (Day 3 treatment only) was the same as the dose used in an earlier report (3). Doses of GZ and MAIQ–1 which would cause minimal toxicity as estimated from data from Screening Data Summary Sheets were selected (we are grateful to Dr. M. Benton Naff, Drug Synthesis and Chemistry Branch, DTP, DCT, NCI, for furnishing us with copies of these screening data from various screening laboratories). Dosages, vehicles, and related information are shown in table 1. Survivors were tabulated twice daily; mean weights of all groups were recorded on Days 3 and 8. Mice had constant access to Wayne Lab-Blox, and the drinking water contained 0.025 mg tetracycline/ml. Data were expressed as treated/control (T/C) survival times × 100 or as % increase in lifespan (% ILS), which is numerically equivalent to (T/C × 100) – 100. Observations were terminated on Day 60; all mice surviving at that time were recorded as "cured," but were included in calculations of T/C as having survived only 60 days. Since the mean survival time of all control mice (720 mice) was 175 ± 8 hours (7.3 ± 3 days), the T/C values have a built-in upper limit of about 820.

RESULTS

The collective results of treatment on Day 3 with each Pt compound and with CY are shown in table 2. For each Pt compound, these collective data represent six groups of ten mice each. For CY, the collective data represent 18 groups of ten mice each. The small standard deviations reflect the high degree of reproducibility of this model. It should be noted that there were no > 60-day survivors with

TABLE 1.—Drugs, doses, and vehicles

Drug	Dose (mg/kg) Day 3 only	Dose (mg/kg) Days 3–6	Vehicle	Comments
cis-DDP	2.5	—	0.9% NaCl	Soluble
NSC-224964	10.0	—	5% glucose	NaCl would tend to regenerate the dichloro analog; was briefly sonicated to facilitate dissolution
NSC-250427	2.0	—	5% glucose	Soluble
NSC-271674	5.0	—	1% $NaHCO_3$	Soluble; forms sodium salt via free carboxyl group
CY	50	—	0.9% NaCl	Soluble
HU	1000	250	0.9% NaCl	Soluble
GZ	1600	400	0.9% NaCl	Fine suspension at higher concentration
MAIQ-1	50	12.5	0.9% NaCl	Fine suspension

any single-drug therapy due to the relatively low doses selected.

The results of Day 3 and Days 3–6 treatment with each of the ribonucleoside diphosphate reductase inhibitors are shown in table 3. Each value represents the mean T/C ± 1.0 SD as calculated from four groups of ten mice each. The greater effectiveness of HU and GZ when given as multiple daily injections is readily apparent, is statistically highly significant, and is totally concordant with anticipated results based upon the relatively rapid urinary excretion of these drugs. However, MAIQ–1, with a biologic half-life approximating 50 hours, was as effective at a dose of 50 mg/kg given as a single injection as it was at a dose of 12.5 mg/kg given as four daily injections. Furthermore, the T/C of 143 obtained at a dose of 12.5 mg/kg is virtually the same as that reported earlier (T/C = 148) by other investigators (16). There were no > 60-day survivors among any of the animals treated with HU, GZ, or MAIQ–1 alone.

The data obtained with the three-drug regimens are compared with the various Pt plus CY combinations in tables 4 (HU), 5 (GZ), and 6 (MAIQ–1). In lieu of ± 1.0 SD values, tabulations of degrees of significance of differences among the groups are included. The inclusion of HU (table 4) as a single large dose on Day 3 significantly increased the mean lifespan of the mice compared to the dual Pt plus CY regimens in all cases. With three of the four Pt compounds, when the same total dose of HU was given as four daily injections there was no therapeutic advantage over the dual Pt plus CY combinations. Substantially the same pattern was observed when GZ was substituted for HU (table 5); the only variations were evident in the levels of significance when comparing groups A versus C and B versus C in the *cis*-DDP series of experiments. When MAIQ–1 was substituted for HU (table 6), the pattern obtained was somewhat more erratic; with *cis*-DDP, the single-injection regimen offered no advantage over the two-drug combination. With the other three Pt compounds, the single injection of MAIQ–1 gave unequivocally superior results compared to the Pt-CY dual regimen. Comparison of group A versus C showed statistically significant

TABLE 2.—Effects of a single (Day 3) injection of each Pt compound and of CY on survival times of BDF_1 mice bearing advanced L1210 leukemia*

Drug	T/C
cis-DDP	159 ± 6
NSC-224964	177 ± 12
NSC-250427	181 ± 6
NSC-271674	146 ± 19
CY	186 ± 11

*Values are expressed as T/C survival times × 100 ± 1.0 SD. *See* table 1 for doses.

TABLE 3.—Effects of single (Day 3) and multiple (Days 3–6) treatment of advanced L1210 leukemia in BDF_1 mice using 3 different ribonucleoside diphosphate reductase inhibitors*

Drug	Treated on Day(s) 3	Treated on Day(s) 3–6	*P* value
HU	113 ± 1	144 ± 5	< 0.005
GZ	110 ± 5	135 ± 9	< 0.025
MAIQ-1	132 ± 14	143 ± 10	NS†

*Values are expressed as T/C survival times × 100 ± 1.0 SD. *See* table 1 for doses.

†NS = not significant at the 95% confidence level.

TABLE 4.—Effects of HU at 2 dose schedules on the prolongation by Pt + CY of survival times of BDF_1 mice bearing L1210 leukemia*

Group	Drugs	T/C	*P* value A vs B	*P* value A vs C	*P* value B vs C	No. of cures/ 10 mice
A	*cis*-DDP + CY	357				1
B	*cis*-DDP + CY + HU, Day 3	655	< 0.005	< 0.025	NS†	5
C	*cis*-DDP + CY + HU, Days 3–6	587				4
A	NSC-224964 + CY	371				1
B	NSC-224964 + CY + HU, Day 3	654	< 0.001	NS	< 0.001	7
C	NSC-224964 + CY + HU, Days 3–6	293				0
A	NSC-250427 + CY	374				1
B	NSC-250427 + CY + HU, Day 3	666	< 0.01	NS	< 0.001	6
C	NSC-250427 + CY + HU, Days 3–6	312				0
A	NSC-271674 + CY	455				2
B	NSC-271674 + CY + HU, Day 3	843	< 0.001	NS	< 0.001	9
C	NSC-271674 + CY + HU, Days 3–6	525				2

*Values are expressed as T/C survival times × 100. *See* table 1 for doses.
†NS = not significant at the 95% confidence level.

differences with three of the four Pt compounds, and in only one series, again with *cis*-DDP, was the four-injection regimen of MAIQ–1 superior to the single-injection protocol.

Cures (> 60-day survival) are tabulated individually in tables 4–6 and are summarized in a collective manner in table 7. The cures show the considerable superiority of the single-injection regimen of HU and GZ when combined with Pt plus CY, even though the single-injection regimens of HU or GZ, when used without Pt plus CY, were definitely inferior to the multiple-injection regimens (table 3). With MAIQ–1 as the third drug, the collective cure rate was uninfluenced by the dosage schedule.

In view of the fact that HU or GZ, when added to various Pt plus CY combinations, is more effective in a single large injection than in repetitive doses, the weight changes of the mice, which may be taken as some measure of toxicity, are of particular interest. These changes are shown in table 8 and reflect the mean weight changes between Days 3 and 8. With HU and MAIQ–1, the mean % weight loss was clearly greater when each was given in a divided-dose regimen. The difference was not so marked

TABLE 5.—Effects of GZ at 2 dose schedules on the prolongation by Pt + CY of survival times of BDF_1 mice bearing L1210 leukemia*

Group	Drugs	T/C	*P* value A vs B	*P* value A vs C	*P* value B vs C	No. of cures/10 mice
A	*cis*-DDP + CY	375				0
B	*cis*-DDP + CY + GZ, Day 3	568	< 0.01	NS†	< 0.05	4
C	*cis*-DDP + CY + GZ, Days 3–6	378				0
A	NSC-224964 + CY	359				1
B	NSC-224964 + CY + GZ, Day 3	523	< 0.05	NS	NS	3
C	NSC-224964 + CY + GZ, Days 3–6	425				2
A	NSC-250427 + CY	455				2
B	NSC-250427 + CY + GZ, Day 3	694	< 0.05	NS	< 0.025	6
C	NSC-250427 + CY + GZ, Days 3–6	459				1
A	NSC-271674 + CY	342				0
B	NSC-271674 + CY + GZ, Day 3	742	< 0.001	NS	< 0.001	8
C	NSC-271674 + CY + GZ, Days 3–6	362				0

*Values are expressed as T/C survival times × 100. *See* table 1 for doses.
†NS = not significant at the 95% confidence level.

TABLE 6.—Effects of MAIQ-1 at 2 dose schedules on the prolongation by Pt + CY of survival times of BDF_1 mice bearing L1210 leukemia*

Group	Drugs	T/C	*P* value A vs B	*P* value A vs C	*P* value B vs C	No. of cures/10 mice
A	*cis*-DDP + CY	343				1
B	*cis*-DDP + CY + MAIQ-1, Day 3	344	NS†	< 0.001	< 0.001	1
C	*cis*-DDP + CY + MAIQ-1, Days 3–6	700				7
A	NSC-224964 + CY	330				0
B	NSC-224964 + CY + MAIQ-1, Day 3	652	< 0.001	< 0.05	NS	6
C	NSC-224964 + CY + MAIQ-1, Days 3–6	533				4
A	NSC-250427 + CY	422				3
B	NSC-250427 + CY + MAIQ-1, Day 3	691	< 0.01	NS	NS	8
C	NSC-250427 + CY + MAIQ-1, Days 3–6	479				4
A	NSC-271674 + CY	319				0
B	NSC-271674 + CY + MAIQ-1, Day 3	548	< 0.025	< 0.025	NS	5
C	NSC-271674 + CY + MAIQ-1, Days 3–6	539				5

*Values are expressed as T/C survival times × 100. *See* table 1 for doses.
†NS = not significant at the 95% confidence level.

with GZ. Particularly noteworthy, however, is that the therapeutically superior regimen of HU, ie, as the third drug given as a single large dose, produced less mean weight loss than did the therapeutically inferior multiple-dose regimen.

Since all possible dual combinations of drugs under study were routinely assessed as part of our standard protocol (1–3,25), these data were examined to determine if either CY or Pt, when given with any of the three reductase inhibitors, produced a greater % ILS than would have been expected on the basis of the sums of the % ILS values when each was used as a single agent. No consistent pattern was noted among the four Pt compounds. However, in all experiments, the combination of CY plus any one of the reductase inhibitors yielded % ILS values which were significantly greater than the sums of the % ILS values of the individual drugs when used alone, and the degree of enhancement did not appear to be influenced by the schedule of administration (table 9).

DISCUSSION

The therapeutic synergism of the dual combination of *cis*-DDP plus CY against L1210 leukemia was first detected early in the 1970s by investigators at Microbiological Associates, Inc, the Drug Evaluation Branch, DTP, DCT, NCI, and the Wadley Institutes of Molecular Medicine (26–29). Even though the therapeutic advantage of co-administration compared with single-drug administration was apparent with various treatment schedules, the most effective regimen was a single injection of each drug (29). The above investigators as well as Schabel (30) have reported that *cis*-DDP is synergistic with a number of other antitumor agents with diverse modes of action when used in the treatment of L1210 leukemia.

Of the ribonucleoside diphosphate reductase inhibitors assessed in the present work, HU has been used most extensively in clinical practice. It is active in man, but is not uniquely effective against any particular tumor (31). It is maximally effective against L1210 leukemia when given in fractionated doses, eg, q3hours × 8 on Days 2 and 6 or on Days 2, 6, 10, and 14 (13). The basic pharmacology of GZ was summarized recently by Adamson (32). It has been used clinically only to a limited extent, but is

TABLE 7.—Collective cures obtained with HU, GZ, or MAIQ-1 + all Pt compounds + CY, with the former 3 drugs administered at 2 dose schedules*

Drugs	Collective cures, > 60-day survival (No. of cures/ No. of mice treated)	
Pt + CY	5/40	(13%)
Pt + CY + HU, Day 3	27/40	(68%)
Pt + CY + HU, Days 3–6	6/40	(15%)
Pt + CY	3/40	(8%)
Pt + CY + GZ, Day 3	21/40	(53%)
Pt + CY + GZ, Days 3–6	3/40	(8%)
Pt + CY	4/40	(10%)
Pt + CY + MAIQ-1, Day 3	20/40	(50%)
Pt + CY + MAIQ-1, Days 3–6	20/40	(50%)

*See table 1 for doses.

TABLE 8.—Mean % weight changes of BDF_1 mice bearing advanced L1210 leukemia when treated with Pt, CY, and each of 3 ribonucleoside diphosphate reductase inhibitors, with each of the latter given on Day 3 only as a single large dose or on Days 3–6 in equally divided doses*

Drugs	% weight change			
	cis-DDP	NSC-224964	NSC-250427	NSC-271674
Pt + CY + HU, Day 3	–2	–2	–4	–9
Pt + CY + HU, Days 3–6	–13	–17	–16	–17
Pt + CY + GZ, Day 3	+1	+1	–1	+1
Pt + CY + GZ, Days 3–6	–3	–1	–7	–4
Pt + CY + MAIQ-1, Day 3	–2	–4	–5	–6
Pt + CY + MAIQ-1, Days 3–6	–12	–13	–11	–14

*See table 1 for doses.

considered to be an active agent for remission induction in certain types of adult leukemia (33). MAIQ–1, which has not yet been entered into clinical trials, was designed and synthesized (15) as a potential second-generation antineoplastic agent of the α-(N)-heterocyclic carboxaldehyde thiosemicarbazone class. An earlier derivative, 5-hydroxy-2-formylpyridine thiosemicarbazone, was active against certain transplanted and spontaneous animal tumors, but was not comparably effective in man (34,35). This lack of clinical activity was attributed to a relatively low inhibitory action against the reductase, as well as to its rapid metabolism to an O-glucuronide with resultant rapid excretion. MAIQ–1 contains an amino group in the 5-position rather than an hydroxyl group, thus obviating the possibility of O-glucuronidation, and a 4-methyl group on an isoquinoline ring system which introduces sufficient steric hindrance to minimize inactivation through N-acetylation. These structural modifications yielded a compound which is about 30-fold more active than 5-hydroxy-2-formylpyridine thiosemicarbazone as an inhibitor of ribonucleoside diphosphate reductase activity (16).

In a study which began in 1971, Piel and Perlia (36) treated a total of 74 patients with *cis*-DDP alone or in combination with CY. The overall response rate was 24% (18 of 74 patients). Of the 74 patients, 20 received the combination of *cis*-DDP plus CY; the response rate in this group was 50%

TABLE 9.—Therapeutic response to dual treatment of BDF_1 mice bearing advanced L1210 leukemia with CY + HU, GZ, or MAIQ-1, compared with sums of responses (Σ) with each drug used alone*

Drugs	% ILS ± 1.0 SD	P value
CY + HU, Day 3, Σ	111 ± 20	
CY + HU, Day 3, co-administered	234 ± 70	< 0.05
CY + HU, Days 3–6, Σ	129 ± 23	
CY + HU, Days 3–6, co-administered	188 ± 13	< 0.01
CY + GZ, Day 3, Σ	99 ± 17	
CY + GZ, Day 3, co-administered	183 ± 28	< 0.005
CY + GZ, Days 3–6, Σ	109 ± 4	
CY + GZ, Days 3–6, co-administered	224 ± 59	< 0.025
CY + MAIQ-1, Day 3, Σ	113 ± 22	
CY + MAIQ-1, Day 3, co-administered	242 ± 54	< 0.01
CY + MAIQ-1, Days 3–6, Σ	137 ± 12	
CY + MAIQ-1, Days 3–6, co-administered	202 ± 17	< 0.005

*Data are presented as % ILS [(T/C × 100) – 100] to avoid the arithmetic ambiguity which would be induced by summation of T/C values. *See* table 1 for doses.

(ten of 20 patients). Responders to the dual regimen included cancer patients with sarcoma (four of six patients), head or neck carcinoma (one of two patients), testicular carcinoma (one of one patient), Hodgkin's disease (one of one patient), ameloblastoma (one of one patient), and tumors of unknown primary (two of three patients). The six other nonresponders had cancer of the stomach, colon, or lung. Eagan et al (37) combined *cis*-DDP with CY and adriamycin in the treatment of 41 evaluable patients with advanced non-small cell lung cancer. The initial response rates of the three tumor types were: 44%, squamous cell carcinoma; 43%, adenocarcinoma; and 22%, large cell carcinoma. Two other recent major studies at Roswell Park Memorial Institute included *cis*-DDP in various combinations with adriamycin, bleomycin, vinblastine, and CY (38,39).

As far as we are aware, no clinical study of polychemotherapy with *cis*-DDP, CY, and an inhibitor of ribonucleoside diphosphate reductase has been done. In view of the widely recognized clinical predictability of the L1210 leukemia model (40,41), a clinical protocol of this type seems warranted.

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Important Clinical Pharmacologic Considerations in the Use of Methadone in Cancer Patients [1,2]

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SUMMARY

The manufacturer's recommended dose of methadone, which is 2.5–10 mg every 3–4 hours, is excessive. Methadone has an average primary-phase half-life of 14.3 hours and a slower secondary-phase half-life averaging 54.8 hours. Administering the recommended dose of methadone and escalating doses within 2–3 days may lead to very high plasma levels of the drug. In addition, factors such as drug-drug interaction and/or hepatic and renal dysfunction may affect the metabolism of methadone and result in its accumulation. Methadone is an effective analgesic whose pharmacokinetics must be appreciated in order for it to be used safely and effectively. Three cases of methadone overdose are presented.

[Cancer Treat Rep 63:457–459, 1979]

Methadone is commonly prescribed as a narcotic analgesic for cancer patients with pain. Pharmacologic data available indicate that the dose and frequency of administration of methadone recommended by standard pharmaceutical reference sources are in error and may lead to drug accumulation (1–5). We report here three patients treated with "recommended" doses of methadone. When administered in this fashion extreme somnolence and/or coma resulted.

CASE REPORTS

Patient 1

A 78-year-old black female who had a laryngectomy for carcinoma of the larynx was found to have a poorly differentiated adenocarcinoma of the lung. Because of persistent right chest pain, on August 11, 1978, the patient was given methadone, 3 mg orally every 6 hours. However, instead of this dose, she took 12 mg orally every 6 hours. She was not receiving cytotoxic chemotherapy, but she had been taking sodium levothyroxine and dioctyl sodium sulfosuccinate. On August 23, 1978, the patient was admitted to the hospital; she was unresponsive with depressed respirations (3/minute) and was breathing very shallowly. Liver and kidney function studies were normal except for a mild elevation of the alkaline phosphatase level (50 IU/liter; normal = 10–32 IU/liter). The patient's family observed that for several days prior to admission the patient had developed increasing drowsiness. She was immediately treated with naloxone, 0.8 mg iv, with an immediate response. Her respiratory rate immediately increased to 16/minute and she became alert and oriented. Over the next 24 hours after admission, she lapsed into a stuporous state several times. These episodes were readily reversed by giving 0.4 mg of naloxone iv. By August 26, 1978, she was discharged. She and her family were given explicit instructions on the judicious use of methadone at the prescribed doses.

Comments.—Although the patient took four times the amount of methadone prescribed by her physicians, the dose taken was still in the range recommended by the manufacturers.

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Patient 2

A 21-year-old white male with widespread bone and lung metastasis from Ewing's sarcoma was admitted to the Johns Hopkins Oncology Center on August 4, 1978, with the chief complaint of severe headaches and hip pain. The pain was not relieved by oxycodone and hydromorphone, 4.0 mg every 3 hours. The patient had not received cytotoxic chemotherapy in the preceding 2 months. At the time of admission, he was treated for his pain with methadone, 5 mg orally every 4 hours, with resultant reduction in pain. During the latter part of his hospitalization, in addition to analgesics, he was receiving nafcillin and prochlorperazine. Liver and kidney function studies were normal. On August 28, 1978, however, his pain worsened and the methadone dose was increased to 10 mg every 3 hours. On August 29, a modified Brompton's mixture was begun, consisting of 10 mg of methadone and 10 mg of cocaine per dose given every 4 hours. On August 31, 1978, and September 2, 1978, the amount of methadone in Brompton's mixture was increased to 15 and 17.5 mg respectively. Since his pain was still uncontrolled, the methadone dose was further increased to 20 mg every 4 hours. After six doses the patient became stuporous, with severe respiratory depression and miotic pupils. Naloxone, 0.2 mg iv, was administered and resulted in rapid reversal of respiratory depression and heightened level of consciousness. Subsequent doses of methadone and cocaine were withheld. Two and one-half hours after the naloxone administration, the patient died of a cardiorespiratory arrest. Autopsy revealed extensive tumor involving the bones, lungs, abdominal soft tissues, and brain. In addition, bilateral uncal notching of the brain indicated that the metastases were producing brain stem compression.

Comments.—The administration of increased doses of methadone over a relatively short period of time eventually contributed to the methadone accumulation. Although the toxicity was temporarily corrected with a small dose of naloxone, complete correction of methadone overdose should have been accomplished with larger and more frequent doses of naloxone.

Patient 3

A 59-year-old white female with small cell carcinoma of the lung and chronic lung disease was seen in the Outpatient Clinic of The Johns Hopkins Oncology Center 1 day prior to admission complaining of severe pain and a rash on the right buttock. The patient was found to have herpes zoster in the distribution of the sacral dermatomes. Because of the severe pain of the herpetic lesions, she was treated with methadone, 10 mg orally every 4–6 hours. The patient began her methadone that evening at 5-hour intervals, and after three doses she became weak and unable to move from her bed. Her family brought her to the Outpatient Oncology Clinic where she was found to be stuporous and cyanotic and to have miotic pupils. The patient had last received combination chemotherapy (cyclophosphamide, adriamycin, and VP-16-213) 5 weeks prior to this clinic visit. The only other medication she had been taking regularly was aminophylline. She was admitted to the hospital for evaluation of altered mental status and respiratory insufficiency. Liver and kidney function studies were normal except for a slight elevation of the transaminase level (23 IU/liter; normal = 0–19 IU/liter). At the time of admission, naloxone, 0.4 mg iv, was administered. The patient's respiratory rate and level of consciousness improved. Repeated doses of naloxone were required over the next 8 hours because of recurrence of respiratory depression. Further narcotics were withheld and the remainder of her hospital stay was uneventful. The patient was discharged 8 days after admission.

Comments.—The initial dose of methadone was excessive in this patient and led to life-threatening central nervous system depression. This patient's course illustrates the risks of adhering to the "recommended dose schedule" of methadone in a patient previously unexposed to methadone.

DISCUSSION

Treatment for pain is essential in patients with cancer. Control of pain often requires the use of narcotic analgesics. This class of analgesics carries the risk of central nervous system and respiratory depression, gastrointestinal intolerance, and addiction.

Methadone is a narcotic analgesic which has become increasingly popular for the treatment of pain in patients with cancer. Among the reasons for its popularity are good gastrointestinal absorption, modest degree of gastrointestinal intolerance, and a prolonged half-life which permits less frequent administration. The manufacturer's recommended dose of methadone for oral or parenteral administration in adults is 2.5–10 mg every 3–4 hours. In clinical practice, however, a variety of dose schedules are sometimes employed.

The metabolism and pharmacokinetics of methadone have been studied by many investigators (1–5). Methadone is 85% bound to plasma proteins (6). The drug is localized in the lung, liver, kidney, and spleen. Only a small fraction of the drug enters the brain and the primary site of metabolism is the liver (6).

Clinical studies show that the average plasma half-life of methadone is 25 hours with a range from 13 to 47 hours (1,2,4). The pharmacokinetics of methadone are different in patients newly treated with the drug compared to patients who have been using the drug repeatedly (4,5). Verebely et al (4) and Berkowitz (5) reported that in patients without prior methadone treatment, there is a biexponential plasma decay of the drug with an average primary-phase half-life of 14.3 hours and a slower secondary-phase half-life averaging 54.8 hours. With repeated methadone administration there seems to be a monoexponential plasma half-life of approximately 22.4 hours (4,5). The slow secondary-phase half-life seen after single-dose administration indicates the presence of a depot compartment in which methadone is tightly bound and released at a slower rate than that from the compartment represented by the primary-phase half-life (4). An increase in the methadone dose results in a gradual increase in the plasma concentration (4). Equilibration and "steady-state" plasma levels occur 4–5 days after the new dose is begun (4).

There appears to be no correlation between methadone plasma levels and analgesic response. The analgesia experienced after oral administration of methadone peaks between 1 and 2 hours whereas the peak plasma level occurs between 4 and 6 hours (5). In addition, when peak plasma levels of methadone are reached (6 hours) maximal analgesia has fallen by at least 75% (5). Unlike meperidine and morphine, which have half-lives of 3.2 and 2.6 hours respectively (7), methadone has a prolonged plasma half-life. Like meperidine and morphine, however, the analgesia produced by methadone lasts 3–5 hours (6). Since the half-life of methadone is prolonged, frequent dose escalations and short dose intervals will lead to increasing methadone accumulation which may result in toxic plasma levels.

When prescribing methadone, a number of factors which may affect the plasma half-life of the drug and the patient's response to the drug must be considered: (*a*) The possibility exists of drug-drug interaction between methadone and other drugs administered concurrently. These interactions may involve alteration in protein binding, metabolism, and excretion of methadone. In particular, drugs which influence the pH of the urine will alter the excretion rate of methadone (1). (*b*) Hepatic and renal dysfunction will tend to increase the half-life of methadone. (*c*) The age of the patient may be important since the elderly do not tolerate opiates well, although very little is known about methadone metabolism in older patients (8). (*d*) Patients who are opiate-naive tend to have an exaggerated response to initial doses of methadone.

Narcotic antagonists such as naloxone are effective for the treatment of opiate-induced respiratory depression. Naloxone may be administered sc, im, or iv at recommended doses of 0.4–0.8 mg. A prompt reversal of the depressive effects of methadone is usually seen within 2–3 minutes after naloxone is administered. The duration of the antagonistic effect depends on the dose and route of administration of naloxone and usually lasts 1–4 hours (6). Patients who are treated with naloxone and have a favorable response should be observed carefully, and repeated doses of naloxone should be administered as needed. In many instances, as in the case of methadone, the narcotic has a longer duration of action than naloxone.

With the increased use of methadone as an analgesic for the treatment of pain in patients with cancer, the chance of drug accumulation with increasing methadone plasma levels and overdosage becomes an increasing problem. The manufacturer's recommended doses of methadone as written in the *Physicians' Desk Reference*, if interpreted as reasonable starting doses, may be potentially lethal. The adult dose of 2.5–10 mg given every 3–4 hours, however, would be a tolerable range of dose administration if the lower doses were given initially allowing for a degree of drug tolerance to develop. If doses of methadone are increased it must be kept in mind that the plasma level of the drug will steadily rise for about 4 days before reaching a plateau level. Monitoring methadone plasma levels intermittently at various dose escalations may alert physicians to possible toxicity. In each case, doses and/or dosing intervals of methadone should be adjusted accordingly.

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The Role of Autologous Stem Cell Reconstitution in Intensive Therapy for Resistant Neoplasms [1]

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Since the introduction of single-agent chemotherapy for the management of malignant diseases, combination chemotherapy regimens incorporating multiple agents with non-overlapping toxic effects have been used to produce complete remissions in significant numbers of patients. This principle of therapy and that of intensive combined-modality therapy were first applied successfully to the treatment of hematopoietic neoplasms (1–5). Subsequent experience has proven the applicability of this approach to nonhematopoietic neoplasms as well (6–9). Unfortunately, complete remissions are frequently of short duration. Accordingly, a number of centers are now studying the efficacy of more intensive treatment regimens which employ combinations of chemotherapy with or without irradiation in an effort to increase both the frequency and durability of complete remissions among patients with leukemia, lymphoma, and solid tumors of the lung, ovary, and testes. The increased morbidity of such regimens has been due in large measure to pronounced myelosuppression with ensuing prolonged periods of granulocytopenia and thrombocytopenia. Protected environments and hematopoietic support have been utilized to reduce the risk of such therapy. The role of platelet support (10), protected environments (11), prophylactic and therapeutic granulocyte transfusions (12–15), and pharmacologic rescue of hematopoietic cells (16) has recently been reviewed. These efforts are, in general, predicated on the assumption that the regenerative capacity of the marrow cells has not been irreversibly destroyed by either the primary disease or the treatment designed to effect its cure.

The potential morbidity and mortality of intensive treatment regimens designed to eradicate leukemia cells following relapse or demonstrated resistance to conventional-dose therapy suggested that acceleration of hematopoietic recovery by reconstitution with allogeneic or autologous stem cells would reduce the risks of intensive therapy and permit the safe administration of therapy incorporating total-body irradiation and high-dose chemotherapy (4,5,17). These considerations led to the first use of infusions of marrow containing allogeneic or isogeneic hematopoietic stem cells, and in some cases this treatment resulted in long-term complete remissions in patients with advanced leukemia and lymphoma resistant to less intensive therapy (4,5,17). The success of intensive therapy followed by transplantation with marrow from sibling donors matched at the HLA–A, -B, and -D loci in leukemia patients has been limited by the morbidity and mortality of acute and chronic graft versus host disease, graft rejection, immunosuppression, infection, interstitial pneumonitis, and recurrence of leukemia (4,5,18–20). Nevertheless, treatment of refractory leukemia with high-dose cyclophosphamide and total-body irradiation followed by allogeneic or isogeneic bone marrow transplantation has clearly produced a number of long-term survivors (4,5). Moreover, this experience has shown that therapy which is lethal for resistant, neoplastic hematopoietic cells can be administered without irreversible toxicity to susceptible normal tissues and is followed by prompt hematopoietic recovery if marrow stem cell infusions are utilized. In principle, one would like to apply these principles of leukemia therapy to the treatment of resistant solid tumors by using the patient's own marrow cells to effect hematopoietic reconstitution—thereby avoiding the complications of allogeneic bone marrow transplantation or profound hypoplasia. In this review, the development of the expanding field of autologous hematopoietic reconstitution will be discussed in order to bring the reader abreast of recent progress and developments.

BACKGROUND

The successful use of intensive therapy and autologous marrow infusions requires both the choice of appropriate clinical settings and the presence of the technical capability to collect and preserve

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adequate numbers of hematopoietic stem cells. Moreover, these preparations must be preserved in a viable state until needed (months or years later). Centers engaged in this technique should be able to monitor collections for viability and engrafting potential both before and after cryopreservation. The choice of the appropriate indications for the use of autologous marrow infusions is based on the following considerations: (*a*) The neoplasm in question must be demonstrably more sensitive to higher doses of therapy than to conventional-dose therapy. (*b*) The therapeutic regimen used must be effective at a dose which produces tolerable toxicity to nonhematopoietic tissues. (*c*) The choice of agents for combination chemotherapy should not be based upon their degree of toxicity for hematopoietic cells but upon the extent of their activity against the tumor. (*d*) Since hematopoietic recovery from stem cells contained in bone marrow infusions requires a minimum of 14–21 days to begin to achieve full reconstitution of peripheral blood counts, autologous marrow infusions should be utilized only in association with regimens in which profound myelosuppression persists for periods of > 2–3 weeks. Finally, (*e*) the collection of the hematopoietic stem cells from the patient should be carried out before the institution of intensive therapy, and in such a way as to minimize the probability that cancer cells are contaminating the normal hematopoietic cells from the patient.

TECHNICAL ASPECTS OF THE CRYOPRESERVATION OF NORMAL AUTOLOGOUS HEMATOPOIETIC CELLS

As outlined above, the collection of stem cells from patients must be scheduled at a time when the chance for contamination with cancer cells is minimized. This time is assumed to occur when the marrow is morphologically free of tumor cells, when the body burden is at its lowest point (sometime following the achievement of the first complete remission), and before resistance to conventional-dose chemotherapy emerges. Since an interval of time elapses between collection and infusion, if one intends the intensive therapy and autologous reconstitution to occur following the development of relapse and resistance to conventional-dose therapy, some means of long-term storage of the hematopoietic cells must exist. In fact, storage is usually necessary even when the infusions of autologous hematopoietic cells are to be used in the support of intensive therapy in the adjuvant setting, since the treatment regimens often require > 2 and up to 10 days to administer. As early as 1952 such considerations led to the development of methods for the cryopreservation of marrow cells (21). Glycerol was introduced as the first cryoprotective agent (22) but has been largely supplanted by dimethylsulfoxide (23–33). The development of the methods now used for the cryopreservation of human marrow has been well-reviewed by Pegg (34) and Shlafer (35). The cooling rate associated with the survival of the greatest number of viable stem cells has been determined to be 1°C/minute. There is an optimal cooling rate for each type of cell in the marrow (36,37). Cooling at 1°C/minute promotes the survival of stem cells and results in the lysis of terminally differentiated cells (eg, polymorphonuclear leukocytes). Cooling at a controlled rate should be continued to a temperature of −40°C to −50°C before transferring the preparation to storage in liquid nitrogen. Human myeloid and erythroid precursor cells (38,39) survive freezing and thawing in this manner and stem cells survive for at least 100 weeks (40) when appropriately cryopreserved and immersed in liquid nitrogen.

Since most workers have found that a cooling rate of 1°C/minute is optimal for stem cell survival, controlled-rate freezers have been utilized. These devices provide for cooling at what is predetermined to be the optimal rate and also protect cells from rewarming when the heat of fusion is released at the time of the change from the liquid to the solid phase (41,42). Murine marrow samples, when infused following 2 years of storage, have been observed to lead to full hematopoietic reconstitution following total-body irradiation (40). Storage in the vapor phase of liquid nitrogen has been associated with long-term survival of stem cells, while cryopreservation at higher temperatures has resulted in significant losses in the number of viable stem cells remaining after thawing (28,40,41,43).

ANIMAL MODELS FOR THE STUDY OF AUTOLOGOUS MARROW RECONSTITUTION

An important step in the field of hematopoietic reconstitution was the development of animal models which were used to assess the reconstitutive capabilities of samples of marrow cryopreserved by different methods. The model for these experiments came from the early work of Barnes and Loutit (23) in which survival for 30 days following total-body irradiation was used as an endpoint for analysis of animals so treated in the presence or absence of infusion of preparations of hematopoietic stem cells. In experiments utilizing allogeneic bone marrow transplantation, chromosomal or enzymatic markers were available to identify donor and recipient

cells. The documentation of engraftment in autologous marrow infusions however, relied on the comparison of the kinetics of recovery in animals receiving or not receiving infusions of autologous marrow cells following intensive therapy, or on the survival of animals with or without autologous reconstitution following lethal therapy such as total-body irradiation. Initial studies in dogs by Mannick et al (44), which showed that seven of eight dogs exposed to 1500 roentgens of total-body irradiation and given unfrozen autologous marrow infusions survived for > 60 days, were extended later by Gorin et al (45). These latter workers exposed dogs to 1000 rads of total-body irradiation, supported all animals with fluids, antibiotics, and platelet infusions, and showed that six of six dogs given 1000 rads of total-body irradiation without marrow infusions had died by Day 12 following the irradiation, whereas all of ten dogs given $\geq 0.5 \times 10^8$ nucleated cryopreserved autologous marrow cells per kg of recipient body weight survived > 80 days after irradiation and transplant (45). Canine models based upon delivery of lethal doses of chemotherapeutic agents have also been established to test the efficacy of autologous cryopreserved infusions (46).

Data acquired in the course of rodent work showed that the minimum dose of unfrozen nucleated marrow cells per kg of recipient body weight needed to achieve 100% survival of animals following at least 900 roentgens of total-body irradiation (47) was 0.6×10^8. A similar dose of 0.5×10^8 nucleated cryopreserved autologous marrow cells per kg was established for dogs treated with total-body irradiation by Gorin et al using a similar model (45). Data collected with infusions of fresh, unfrozen autologous marrow cells in rhesus monkeys following 900 rads of total-body irradiation suggested that 0.4×10^8 nucleated cells per kg were needed for survival after total-body irradiation (48). The doses of autologous cells reported above, which were required to ensure survival of 100% of animals exposed to lethal doses of total-body irradiation, were much lower than the doses of nucleated marrow cells ($3–7 \times 10^8$/kg) found to be necessary for survival in similar animal models, when the hematopoietic cells came from an allogeneic donor (49). This model of total-body irradiation and reconstitution with cryopreserved autologous marrow cells has also been used to study the effect of the dose of nucleated marrow cells infused on the rapidity with which marrow engraftment occurs (45,50).

Another major technical advance which promoted the development of this field was the creation of in vitro assays which could be used for measurement of the content of viable stem cells in marrow preparations. The first step in the development of these assays was the observation by Till and McCulloch (51) that infusion of hematopoietic cells into lethally irradiated mice led to the appearance of splenic excrescences. These structures (called spleen colonies or CFU_s) contain all the normal hematopoietic elements (51). Subsequently, Dicke et al (52) established in mice that there is a linear relationship between the content of CFU_s in fractions of marrow and the number of colonies of committed myeloid macrophage precursor cells (CFU_c) which can be cultured in vitro in semisolid culture media (52). Such assays as these have permitted workers to establish that the optimal conditions for cryopreservation worked out previously for marrow cells in terms of yield of total nucleated cells were also promoting the survival of the hematopoietic precursor cells which are found to be in the marrow at far lower frequencies than the nucleated myeloid and erythroid cells (38,39). Subsequent studies in dogs have shown that stem cells found circulating in the peripheral blood can also be successfully cryopreserved for reconstitution.

Dimethylmyleran is an agent which manifests its toxicity in the dog in the dose range of 7–10 mg/kg as profound hematopoietic depression, while at 15 mg/kg, serious gastrointestinal toxicity develops (46). Kolb et al (46) treated ten dogs with 7.5 mg/kg of dimethylmyleran without marrow infusions and observed that within 17 days following treatment all of the animals died profoundly neutropenic of either bacterial sepsis, pneumonia, or bleeding. In contrast, eight of 11 dogs treated with 7.5 mg/kg and all of seven dogs treated with 10 mg/kg of dimethylmyleran survived following infusions of their cryopreserved autologous marrow cells (46). A similar protective effect of cryopreserved autologous marrow cells has been observed in dogs following treatment with high doses of cyclophosphamide (53) and in mice (54) given high doses of thioTEPA (5 mg/kg), followed by infusion of unfrozen isogeneic marrow cells.

Experimentation in rhesus and stump tailed monkeys has also suggested that infusions of cryopreserved autologous marrow can be life-saving to animals exposed to lethal doses of chemotherapy or irradiation (41,55–58). Buckner et al (55) established a model in which monkeys were given dimethylmyleran and were supported with fluids, blood, antibiotics, and platelet infusions (55). All of 11 animals given 10 mg/kg of dimethylmyleran died either of massive bloody diarrhea (within 11 days of treatment) or of profound neutropenia and thrombocytopenia (from 11 to 19 days following treatment). Ten additional animals were given 10 mg/kg of dimethylmyleran followed by infusions of their cryopreserved autologous marrow cells ($3–9 \times 10^8$ nucleated

marrow cells per kg of body weight). Eight of the ten animals treated in this manner survived for > 60 days despite the occurrence of massive and bloody diarrhea in this group (55). Thus, in this model, infusions of autologous marrow cells were capable of protecting animals from the lethal myelosuppressive effects of dimethylmyleran, as measured by survival > 60 days following treatment.

Rhesus monkeys given 1200 rads of total-body irradiation have also been studied by Buckner et al for survival in the presence or absence of infusions of autologous cryopreserved marrow cells (41). All four animals given irradiation without autologous marrow infusions died within 13 days following irradiation, while four of six animals receiving infusions of marrow stored for 4 hours at 4°C survived (41). A total of eight of 20 animals receiving cryopreserved autologous marrow infusions (stored at −80°C to −170°C) survived > 60 days. Although there may have been factors which were not under control in these studies which resulted in the death of a number of autografted animals, a definite protective effect of cryopreserved marrow infusions in monkeys was demonstrated (41).

A model has also been reported using stump tailed monkeys in which a protective effect of unfrozen autologous marrow infusions has been observed following total-body irradiation (56–58). In contrast to the above studies, no measurable protective effect of autologous marrow infusions has been observed in primates following administration of high-dose cyclophosphamide (59). Rhesus monkeys not receiving marrow infusions exhibited survival and relatively mild hematopoietic depression following doses of cyclophosphamide which ranged up to 200 mg/kg (59). The majority of animals treated with 240 mg/kg died with or without infusions of cryopreserved autologous marrow cells due to massive hemorrhage in the epicardium, myocardium, liver, bladder, adrenals, and gastrointestinal tract (59). Thus, the potential value of autologous hematopoietic reconstitution in the support of intensive therapy appears to depend in part on the selective toxicity of the agent used for hematopoietic cells. The ultimate utility of intensive therapy and autologous stem cell support will be measured by the degree to which the tumor cells share with the marrow a sensitivity to the treatment that is greater than the other normal tissues of the body. These animal experiments have demonstrated unequivocally that cryopreserved autologous marrow cells can rescue animals from the otherwise lethal myelosuppression which follows high-dose total-body irradiation. The data from these animal models also show that the differential sensitivities of each agent at each dose to be used must be known for each species of animal in which it is to be used in order to predict the feasibility of using infusions of cryopreserved autologous hematopoietic stem cells to protect animals from the toxicity of intensive therapy.

CLINICAL STUDIES OF AUTOLOGOUS MARROW INFUSIONS IN SUPPORT OF INTENSIVE THERAPY IN MAN

The initial experience necessary to develop the technical aspects of marrow collection and to establish that suspensions of marrow cells, filtered so as to remove aggregates of fat and bone spicules, could be safely infused in man came from experience gained in allogeneic bone marrow transplantation (60,61). Some of these early experiences in patients following either irradiation or chemotherapy involved marrow suspension from ribs removed from healthy individuals or from cadavers (60). Subsequent experience involved marrow suspensions collected from the posterior and anterior iliac crests (61) by multiple percutaneous aspirations. Following filtration through stainless steel mesh screens and concentration, the marrow was mixed with a cryoprotectant and frozen by use of a programmed freezer which regulated the rate of cooling and prevented rewarming during the release of heat which occurs at the time of phase change (62–65). Other experience in dogs with cryopreserved buffy coat preparations demonstrated that most of the precursor cells survive the freezing and thawing, while the majority of terminally differentiated cells, both of erythroid and myeloid origin, lyse during thawing (66). The release of nucleoprotein from these mature cells which have lysed is associated with the formation of a gel which may entrap the surviving stem cells if the preparation is allowed to stand too long subsequent to thawing and prior to reinfusion (66). Some investigators have attempted to avoid this problem by fractionation of the marrow before freezing so as to remove most of the mature myeloid cells (67), or by preventing clumping after thawing by producing a slightly acid pH (67). Other workers have used unfractionated marrow by thawing it quickly at the bedside immediately prior to reinfusion (62–65). Using this approach toxic side effects result only when the infusion is delivered too rapidly (60,62–65,68–70). These toxic side effects include headache and substernal pressure. Other minor side effects include burning at the site of infusion, transient hemoglobinemia and hemoglobinuria (due to the lysis of erythrocytes during the thawing of the stored marrow cells), and loss of appetite as well as a mildly unpleasant taste due to

the presence of dimethylsulfoxide (60,62–65,68,69). Thus, cryopreserved autologous marrow cells can be infused safely without major morbidity.

Although the experience in animal models as summarized above clearly demonstrates that autologous marrow infusions accelerate hematopoietic recovery following intensive therapy, the early experience in man (68–70) with unfrozen (69) or frozen (68,70) autologous marrow cells was inconclusive in this regard due to the absence of control groups of patients receiving identical therapy but not receiving marrow infusion. The absence of a control group in which marrow infusions were not used did not permit these early workers to compare the kinetics of hematopoietic recovery with or without marrow infusions. The experience gathered in isogeneic bone marrow transplantation following therapy (1000 rads of total-body irradiation plus 120 mg/kg of cyclophosphamide) which was sufficiently intensive to destroy all endogenous hematopoietic function had shown that infusions of hematopoietic cells in numbers sufficient for engraftment did not result in recovery of peripheral blood counts until 14–21 days following irradiation (71). In addition, therapy intensive enough to require some form of exogenous reconstitution usually is accompanied by a depression of the peripheral wbc count to $< 200/mm^3$ and the platelet count to $< 20,000/mm^3$ (4,62,71,72). In retrospect, early attempts to study the effect of infusions of non-cryopreserved autologous marrow cells following mechlorethamine therapy for solid tumors utilized doses of chemotherapy which were too low since the platelet counts never fell to $< 50,000/mm^3$ (68,69). These early studies also utilized doses of infused marrow which were $< 0.5 \times 10^8$ nucleated cells per kg of recipient body weight, which had been found necessary for reconstitution in the animal models discussed above (45–48). Thus, when studies were performed in man with mechlorethamine therapy in two groups of patients, one receiving and one not receiving cryopreserved autologous marrow infusions, no difference was noted in the kinetics of hematopoietic recovery (73). The first evidence in man that cryopreserved autologous marrow suspensions were effective when given in adequate doses (usually thought to be $\geq 0.5 \times 10^8$ nucleated cells per kg of recipient body weight) was reported by Clifford et al (74). These workers used mechlorethamine as palliation for patients with inoperable solid tumors in Uganda where radiation therapy was unavailable (74). They found that all of the patients treated with mechlorethamine at 2.0 mg/kg given without autologous marrow infusion died shortly after treatment, while the same treatment followed by cryopreserved autologous infusions resulted in survival and partial tumor responses in all three patients treated (74). Pegg et al (70) reported that bone marrow biopsies taken from the irradiated fields in patients receiving infusions of cryopreserved autologous marrow cells following high-dose wide-field irradiation exhibited recovery of marrow cellularity in the radiation field more rapidly than observed in historical controls treated similarly but not receiving marrow infusions (70). In these latter studies, the doses of marrow cells infused were often very low, the treatment delivered and the clinical conditions during treatment were heterogeneous, and no concurrent control groups were utilized (70).

Failure to show an effect of autologous marrow infusions on reducing the morbidity or mortality of a treatment regimen, or on the rapidity of hematopoietic recovery in man, has occurred in at least two reports from the fact that even very intensive doses of drugs (cyclophosphamide followed by cryopreserved autologous marrow cells [75] or hydroxyurea followed by infusions of unfrozen autologous marrow cells [76]) did not result in sufficiently profound or prolonged depression of the peripheral granulocyte and total wbc counts. Therefore, with these two drugs used as single agents, nonhematopoietic toxicity may limit the degree to which doses may be increased and thus no indication exists for marrow infusions with these agents when used alone as single agents (75,76).

Several more recent reports of autologous marrow infusions in the treatment of advanced nonhematologic malignancies (77,78) in which adequate doses of infused marrow cells were utilized are difficult to analyze because of the absence of data showing the kinetics of recovery of patients given a similar treatment without marrow infusions. The unfrozen suspensions of marrow cells which were used in the first of these studies (77) have been shown by these workers to be viable at 4°C for 48 hours. However, these studies reported partial responses in patients whose disease had been shown to be resistant to standard dose chemotherapy (77,78). Thus, although the contribution of autologous marrow infusions remains unclear in these particular studies, responsiveness to dose escalation was documented. Another recent study by Tobias et al (79) studied the effect of dose escalation of a combination of adriamycin and cyclophosphamide, with each patient used as his own control. Patients were treated with this chemotherapy regimen with and without infusions of cryopreserved marrow. In this manner, these workers attempted to assess the effect of dose escalation on tumor response and the effect of the autologous marrow infusion on the rate of hematopoietic recovery following the intensive chemotherapy. This study failed to show any impact of autolo-

gous marrow infusion on wbc counts in association with this treatment. In spite of their failure to utilize doses of drugs which were sufficiently intensive to require marrow infusion, these workers were able to document partial responses and one complete response in a group of cancer patients (79) whose tumors had been previously resistant to treatment.

Burkitt's lymphoma has been used as a model to test the efficacy of intensive, nonlethal chemotherapy in patients who relapsed and who were shown to be resistant to conventional-dose therapy (62–63,80). In these studies, the effect of cryopreserved autologous marrow infusions on the rate of hematopoietic recovery was also studied by comparing two groups of patients treated identically except that one received infusions of cryopreserved autologous marrow cells and one did not (62,63,80). These studies showed that the median days spent with granulocytes $< 100/mm^3$ or leukocytes $< 1000/mm^3$ in the peripheral blood were 16 and 23 days respectively among patients treated without marrow infusions, and 8 and 13 days respectively in the autografted patients (62,63). The number of days spent with temperatures $> 38.5°C$ was also greater in the non-autografted group but there was no difference with respect to the incidence of gram-negative sepsis or death during the treatment period between the groups receiving or not receiving marrow infusions (80). The treatment regimen resulted in disease-free survivals in three patients of > 29, 19, and 9 months at the time of publication (80). Four of the patients died during therapy, three from treatment-related cardiomyopathy (62,80). All of the remainder of the patients exhibited transient responses to this therapy (62,80). Thus, this experience with intensive chemotherapy, used at the limit of nonhematologic toxicity, showed a definite effect of autologous marrow infusions on the rate at which hematopoietic recovery occurred following intensive combination chemotherapy, and produced three long-term survivors among patients with Burkitt's lymphoma who had relapsed and had been shown to be resistant to conventional-dose chemotherapy (80).

Treatment of nonhematologic malignancies with combined-modality therapy of sufficient intensity so as to be expected to be lethal without hematopoietic reconstitution with cryopreserved autologous marrow has been reported by two groups (81,82). A 39-year-old patient with relapsed small cell carcinoma of the lung was treated with 800 rads of total-body irradiation and intensive doses of adriamycin (150 mg/m^2) and cyclophosphamide (120 mg/kg), as well as high-dose methotrexate with citrovorum factor rescue (81), followed by infusion of cryopreserved autologous marrow cells (1×10^6 CFU_c). Hematopoietic recovery occurred by Day 14 and the patient had a complete remission without maintenance chemotherapy which lasted 5 months (81). This patient later died with widely metastatic disease (81). Three children, whose malignancies were unresponsive to conventional-dose therapy at the beginning of therapy (Burkitt's lymphoma, neuroblastoma, and rhabdomyosarcoma), were given 800 rads of total-body irradiation and intensive combinations of cyclophosphamide and adriamycin followed by infusions of cryopreserved autologous marrow cells (82). All three patients exhibited responses and prompt hematopoietic recovery (82). Other workers are studying non-cryopreserved autologous marrow infusions as a means for the support of intensive combination chemotherapy for solid tumors in adults (83).

Treatment of patients with acute leukemia at the time of relapse with either intensive combination chemotherapy (65) or combined-modality therapy (84) followed by infusion of cyropreserved autologous marrow cells collected during the first remission has resulted in a complete remission of long standing (7 months) in one of eight patients (84). Clearly, the problem confronting the therapist in this situation is the limited ability of the available treatment regimens to achieve sufficient cytoreduction to eradicate leukemia cells completely from the marrow before it is stored at the time of a complete remission. No serologic method has yet been reported which can be shown to further reduce contaminating leukemia cells which may be present in the stored autologous marrow. However, antisera in several laboratories (85,86) exist which exhibit varying degrees of complement-dependent cytotoxicity for leukemia cells under conditions in which little or no toxicity is demonstrable for the normal committed myeloid precursor cells. It should be theoretically possible to use such reagents to reduce the level of leukemia cells which may contaminate marrow isolates from leukemia patients during the first remission.

The treatment of seven patients with chronic myelogenous leukemia in blast crisis has been attempted using combined-modality therapy (1000 rads of total-body irradiation and cyclophosphamide given at a total dose of 120 mg/kg) followed by infusion of hematopoietic cells stored early in the chronic phase of the disease (87,88). Only two of seven patients achieved complete hematopoietic recovery, and these two patients were the only two in the series in whom a combination of peripheral blood and marrow hematopoietic stem cells was used for reconstitution. One of these patients died 72 days following therapy with a fungal pneumoni-

tis and the other died of blast crisis after a 4-month remission (88). It is interesting to note that either incomplete engraftment or incomplete eradication of the blast cells and the limited ability of chronic phase marrow cells to completely reconstitute the marrow of these patients following intensive therapy may account for the disappointing results in these trials (88). A complex regimen of chemotherapeutic agents followed by infusion of cryopreserved buffy coat chronic phase cells has been reported by Goldman et al to result in full hematopoietic recovery in four of five patients and a return to the chronic phase for 1 year in the fifth patient (89). Relapse occurred in one of the five patients at 4 months and followup of the remaining patients was < 3 months at the time of this publication (89).

THE USE OF PERIPHERAL BLOOD CELLS AS A SUPPORT MODALITY IN INTENSIVE THERAPY FOR RESISTANT NEOPLASMS

The existence of circulating hematopoietic stem cells in the peripheral blood has been demonstrated by both in vivo and in vitro experimentation. Hematopoietic recovery has been observed in lethally irradiated rats (90) and baboons (91) following parabiosis of irradiated animals with the blood of non-irradiated animals in a manner that allowed complete mixing of the blood volumes of the animals (90,91). In the case of the rat studies, chromosomal markers were used to locate the presence of the hematopoietic cells of the non-irradiated animal in the marrow of the irradiated animal (90). In vitro assays have also demonstrated the presence of committed myeloid and erythroid precursor cells in the peripheral blood of animals and man (38,66,92–96). Cells which morphologically resemble lymphocytes but lack the surface receptor characteristic of the T and B lymphocytes, and which give rise to the growth of erythroid, myeloid, and megakaryocytic intraperitoneal diffusion chambers, have been reported (93).

These early studies suggested that it would be possible to use cells collected from the peripheral blood of animals and man for purposes of hematopoietic reconstitution following total-body irradiation if adequate numbers of these cells could be collected. There are both practical and theoretic advantages to the collection of stem cells from the peripheral blood. General anesthesia which is usually required for the collection of stem cells from the marrow space in man is not required for peripheral blood collections. Thus, one could consider the application of hematopoietic reconstitution in association with intensive therapy in patients who were not good candidates for general anesthesia or for whom marrow collection of the hematopoietic cells had been precluded by local irradiation delivered to the regions usually chosen for harvesting marrow stem cells. A possible theoretic advantage of autologous hematopoietic reconstitution from the peripheral blood is more complete and rapid immune reconstitution than is possible with marrow cells.

Animal models in the dog have been developed by Bruch et al (66) and Ross et al (97), who have shown that infusions of cryopreserved autologous hematopoietic cells, collected from the peripheral blood by use of the continuous-flow centrifuge (66), can avert the otherwise lethal effect of intensive therapy with total-body irradiation. Debelak-Fehir et al (98) reported that administration of a total dose of 12 mg/kg of busulfan and 50 mg/kg of cyclophosphamide resulted in the death of all of seven dogs within 8 days following completion of therapy. Six of seven dogs treated identically with cyclophosphamide and busulfan, but also given 2.2×10^{10} cryopreserved buffy coat cells following chemotherapy, survived at least 30 days following therapy (98). Several workers have shown that it is possible to rescue dogs from 1000 rads of total-body irradiation by infusion of cryopreserved hematopoietic cells collected from the peripheral blood (66,94,97). In several of these studies (94,99,100), the growth of colonies in semisolid culture media was shown to correlate with the ability to reconstitute dogs from total-body irradiation. These and other workers have shown that 80%–90% of the committed myeloid precursor cells and mononuclear cells collected from the peripheral blood survived the cryopreservation method used for storage of these cells (64,94,97). Cell separators have been used, in man as well as in dogs, to collect peripheral blood hematopoietic precursor cells (66,92,97,100). A series of five 4-hour collections from a single human donor with the continuous-flow centrifuge (92) has been shown to yield 5×10^{10} mononuclear cells and 4×10^{7} CFU_c. Lower numbers of CFU_c ($0.5–1 \times 10^{6}$ CFU_c per single 4-hour collection) have been reported by others using the continuous-flow centrifuge in man (102,103). A variety of methods have been devised for concentrating these buffy coat collections so as not to infuse the recipient with large volumes of medium containing cryoprotectants following thawing (66,97), but a definite advantage to these methods over simple infusions of the thawed buffy coat cells has yet to be established.

Interest in finding ways of amplifying the numbers of circulating CFU_c, and thus by inference the number of pluripotent hematopoietic stem cells present in the peripheral blood, has arisen from the observation that the ratio of CFU_c per total nucleated cells in the peripheral blood has been esti-

mated to range from one tenth to one fiftieth of that in the marrow (94,104). The iv administration of dextran sulfate was shown by Ross et al (97) and Richman et al (105) to result in a fourfold to tenfold elevation of circulating CFU_c in the peripheral blood of dogs (97). Since the mononuclear cell count rose by a factor of two, a selective effect of dextran sulfate on the circulating levels of committed myeloid precursor cells was suggested (97). Whether this amplification of CFU_c arose from a release of increased numbers of CFU_c from the marrow or from some other mechanism is not yet known (97). No evidence is available to show that the increased level of circulating CFU_c signifies an increased reconstitutive capability per mononuclear cell.

Other workers have also observed amplification of levels of circulating CFU_c in man (105). Richman et al studied circulating CFU_c levels before, during, and after recovery of counts in patients receiving therapy with adriamycin and cyclophosphamide (105). In nine of the 14 patients studied, up to tenfold amplification in the number of circulating CFU_c per 2×10^5 mononuclear cells occurred in the peripheral blood 14–21 days following chemotherapy (105). Korbling et al (101) have reported collection of 1.1×10^6 CFU_c in a single 4-hour leukapheresis from an untreated normal human donor. Erythroid, granulocytic, and megakaryocytic precursors appeared in diffusion chambers inoculated with these cells, indicating that committed precursor cells are present for all the lines of differentiation in peripheral blood (93). Estimates of the total number of CFU_c required for engraftment of an adult 70-kg patient range between 1 and 10×10^6 (102,103). The achievement of this dose might involve several 4-hour collections using the continuous-flow centrifuge from the blood of a man to harvest sufficient numbers of cells. Therefore, several workers have sought to identify a means of amplication of levels of circulating hematopoietic stem cells. Whether this amplification will be achieved most easily by polyanions (97,104) or by the recovery phase of chemotherapy (105) is unclear. Studies of the reconstitutive powers of this amplified stem cell compartment must also be carried out in animal models before application to studies in man.

CONCLUSIONS

The cumulative experience in animal models has shown that it is possible to protect animals from the hematologically lethal effects of total-body irradiation and intensive combinations of chemotherapeutic agents by infusion of cryopreserved autologous marrow cells. The use of intensive combined-modality therapy as an adjunctive therapy for high-risk patients in complete remission, or for treatment of relapsed patients resistant to conventional-dose therapy, is currently being studied in many centers in a variety of neoplasms. Such trials, which include autologous marrow infusion, will study the effect of intensive therapy on resistant cancers without the limitation previously imposed by the hematopoietic toxicity of these regimens (106,107). The long-term disease-free unmaintained remissions reported for patients with Burkitt's lymphoma (62,80) suggest that if the tumor is sufficiently sensitive to the therapy utilized, it is possible, at least in certain patients, to collect tumor-free marrow cells while these patients are in remission (80). The studies of therapy for solid tumors in man with total-body irradiation combined with chemotherapy (81,82) show that the preparations of cryopreserved autologous marrow can make administration of such therapy safe. The question of the presence of tumor cells in such collections cannot be ruled out with the methods of detection currently available, and hopefully serologic means of automated tumor cell detection will be developed. The experience in animal models, the summarized experience of intensive therapy for blast crisis of chronic myelogenous leukemia (87,88), and the experience in acute leukemia in man (84) underscore once again the necessity for an intensive regimen to which the tumor is responsive and which is not associated with intolerable nonhematopoietic toxicity. The existence of such regimens for a resistant neoplasm and the feasibility of collecting normal autologous hematopoietic cells uncontaminated with tumor cells at some point in the patient's course following diagnosis, are prerequisites for successful use of intensive therapy with autologous marrow reconstitution for the treatment of such diseases. A final conclusion as to the ultimate utility of both intensive therapy of resistant neoplasms and the role of autologous marrow support in this treatment must await the outcome of the many trials designed to test these questions which are now underway in several cancer centers.

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Therapy for Mouse Tumors and Human Tumor Xenografts With the Antitumor Antibiotic AT-125 [1,2,3]

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SUMMARY

The antimetabolite antibiotic L-(αS,5S)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid (AT-125) showed significant antitumor activity against L1210 and P388 mouse leukemias and the M5076 mouse ovarian tumor. Depending on the schedule of administration, increases in lifespan of > 100% were observed. Activity was observed after ip, oral, or sc inoculation of AT-125 in mice inoculated with L1210 by the ip route. Lewis lung carcinoma and B16 melanoma were not affected by AT-125. The compound was used to treat human tumor xenografts in athymic (nude) mice. The MX-1 mammary tumor regressed when treated with either 8 or 16 mg/kg/day for 10 days, while a dose of 32 mg/kg was toxic. On an every-4-days × 3 schedule there was a marked slowing of MX-1 tumor growth at 50, 100, and 200 mg/kg. The LX-1 lung tumor xenograft growth was slowed significantly by a dose of 32 mg/kg. Growth of colon tumors, CX-1, CX-2, and CX-3, was not affected by AT-125.

[Cancer Treat Rep 63:473–476, 1979]

AT-125 was first detected in fermentation broths by Hanka et al in 1973 (1) in an in vitro prescreen designed to select antimetabolites. The structure was determined from the initial purification by Martin et al (2) and is shown in figure 1. It is water-soluble and stable in aqueous solutions. Antibacterial activity was found to be antagonized rather specifically by L-histidine (3), and recently Cooney and his colleagues (4) demonstrated that AT-125 is a powerful inhibitor of a number of enzymes catalyzing amido-transfer from L-glutamine. In mammalian cells, AT-125 activity has been shown to be antagonized by L-glutamine (5). Antitumor activity against L1210 leukemia was first reported in 1973 (1). Since that time, antitumor studies have been performed in a variety of murine tumors, and several of these tests are reported in the present study. With the addition to the Division of Cancer Treatment, National Cancer Institute (NCI) tumor panel of human tumor xenografts grown in nude mice, additional information has been obtained regarding antitumor activity of this drug before preclinical toxicology was performed. Previously reported cancer chemotherapeutic tests in nude mice have shown a positive correlation with the response in patients to the same drug (6,7), and a number of papers reporting on chemotherapy for human tumor xenografts (8–10) have shown promise for this system as a model to evaluate potential drugs for specific tumor types.

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MATERIALS AND METHODS

Drug.—AT-125, prepared at The Upjohn Co, was supplied by the Drug Evaluation Branch, Developmental Therapeutics Program, Division of Cancer Treatment, NCI. It was dissolved in physiologic

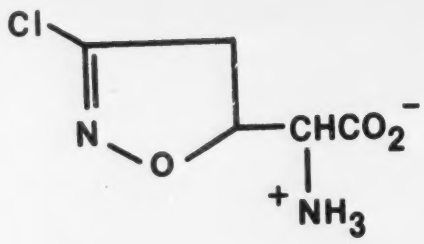

FIGURE 1.—Structure of AT-125.

saline to the appropriate concentration and injected in a volume of 0.01 ml/g of body weight.

Animals and tumors.—Mice were also supplied by the Drug Evaluation Branch of the NCI and were fed food and water ad libitum. Six- to 8-week-old (20–25 g) $B6D2F_1$ (C57BL/6 × DBA/2), $CD2F_1$ (BALB/c × DBA/2), and nude mice of random-bred NIH Swiss background were used in the study. The nude mice were maintained in autoclaved cages under polyester filters and were given autoclaved feed and water adjusted to pH 2.5 with HCl. L1210 and P388 leukemias were maintained in DBA/2 mice while B16 melanoma, Lewis lung carcinoma, and M5076 ovarian carcinoma were maintained in C57BL/6 mice. Standardized protocols of the Developmental Therapeutics Program of the NCI (11) were used in transplanting and testing these tumors. The human tumor xenografts were MX-1, a mammary duct cell carcinoma, CX-1, CX-2, and CX-3, colon adenocarcinomas, and LX-1, an oat cell carcinoma. All of these tumors except CX-1 were obtained from Dr. B. C. Giovanella (Stehlin Cancer Foundation, Houston, Tex). The CX-1 tumor was obtained from a tissue culture established by Dr. J. Fogh (Memorial Sloan-Kettering Cancer Center, Rye, NY). Nude mice were implanted sc with 3-mm^3 tumor fragments in the right subaxillary region. Mice were randomized into test groups when the tumors were 100–300 mg, as determined by caliper measurements, and drug therapy was initiated. The tumor sizes were standardized in the different groups by obtaining relative tumor weight calculated by the formula W_i/W_o, where W_i is the mean tumor weight at any given time and W_o is the mean initial tumor weight when treatment is begun. For evaluation purposes, any treatment group with an average tumor weight of $< 42\%$ of that of the untreated control group was considered responsive to therapy (12), and a relative tumor weight of < 1.0 (the starting tumor size) indicated tumor regression.

RESULTS

Table 1 shows the antitumor spectrum of AT-125 against various murine tumors by different challenge route and by different routes of drug administration. The increase in lifespan (ILS) for ip or sc drug injection against ip L1210 was almost identical while the ILS for oral administration was somewhat lower. Drug activity against ic and sc L1210 was also good. The best activity (150% ILS) was against P388 leukemia, while the drug was ineffective against B16 melanoma and Lewis lung carcinoma.

The effect of AT-125 on the M5076 ovarian carcinoma is seen in the two experiments described in table 2. Doses of 8, 4, or 2 mg/kg/injection were all highly active as seen by total tumor eradication, while a dose of 16 mg/kg/injection was toxic to the mice.

TABLE 1.—Antitumor spectrum of AT-125

Tumor*/ route	Drug route/ schedule	Optimal dose (mg/kg/ injection)	ILS† (%)
L1210/ip	ip/daily, Days 1–9	2–8	65
L1210/ip	oral/daily, Days 1–9	8	36
L1210/ip	sc/daily, Days 1–9	8	62
L1210/ic	ip/daily, Days 1–9	2–8	29–42
L1210/sc	sc/daily, Days 1–9	8	51
P388/ip	ip/daily, Days 1–9	8	150
B16/ip	ip/daily, Days 1–9	8	22
B16/sc	ip/daily, Days 1–9	4	27
Lewis lung/sc	ip/daily, Days 8–16	8	15
Lewis lung/im	ip/daily, Days 5–17	1	1

*6–10 mice/group.
†ILS compared to untreated control group.

TABLE 2.—Effect of AT-125 on growth of M5076 ovarian carcinoma

Experiment No.*	Dose† (mg/kg/ injection)	Mean tumor weight‡	
		mg	T/C§ (%)
1	8	0	0
	4	0	0
	2	27	2
	Vehicle control	909	
2	16	Toxic	—
	8	0	0
	4	0	0
	2	51	3
	Vehicle control	1307	

*10 mice/group.
†Administered ip every 2 days × 11, Days 1–21.
‡Weight 22 days after implant.
§ $T/C = \frac{\text{test animal mean tumor weight}}{\text{control animal mean tumor weight}} \times 100.$

The effects of dose and schedule of administration of AT-125 against L1210 are seen in table 3. Significant ILS values were seen with all schedules except the Day-1 only and the every-8-days schedule. The most effective schedule was with 4 mg/kg/injection on an every-3-hours, every-4-days regimen.

Tables 4–6 show the results of testing against breast, lung, and colon xenografts, respectively. As seen in table 4, there was significant regression of the breast tumor with doses of 8 and 16 mg/kg on a daily × 10 schedule. The dose of 32 mg/kg was toxic. On the every-4-days × 3 schedule there was a marked slowing of the tumor growth with T/C values of 16%, 17%, and 29% for the 200-, 100-, and 50-mg doses, respectively. However, relative tumor weights were never lower than the original tumor size. The tumors in all mice in these experiments ultimately re-grew. AT-125 at a dose of 32 mg/kg on a daily × 9 schedule was very effective against the LX-1 lung tumor (table 5) in slowing tumor growth (T/C, 21%). In tests in the three colon tumors (table 6), there was no effect with any schedule or dose tested against any tumor.

DISCUSSION

The results of murine tumor therapy with AT-125 show a range of activity against P388 and L1210 leukemias as well as M5076 ovarian carcinoma. Antitumor activity was also seen against a human breast and lung tumor xenograft but not against colon tumor xenografts. Similarly, there was no activity against murine colon tumors (unpublished data). Antitumor activity is related to dose and schedule in the various tumor systems. The use of human tumor xenografts as predictors of therapeutic response in man is presently being evaluated by the NCI and the ultimate utility of this system must await results of ongoing experiments in several laboratories. Based on these studies, AT-125 is now undergoing preclinical toxicology studies.

TABLE 3.—Effect of dose and schedule of AT-125 against ip L1210 in mice*

Schedule	Optimal dose (mg/kg/injection)	ILS† (%)
Daily, Days 1–9	8	65
Day 1	128	17
q3hrs, Day 1	32	36
q8days, Days 1 and 9	128	19
q3hrs, Days 1 and 9	8	62
q4days, Days 1, 5, and 9	64	43
q3hrs, Days 1, 5, and 9	4	104

*6 mice/group.
†ILS compared to untreated control group.

TABLE 4.—Effect of AT-125 against human breast (MX-1) tumor xenografts in nude mice*

Treatment schedule	Dose† (mg/kg/injection)	Lowest relative tumor weight‡	T/C§ (%)
Daily × 10	32	Toxic	Toxic
	16	0.63	2
	8	0.76	3
	0	20.23	—
q4days × 3	200	3.11	16
	100	3.32	17
	50	5.52	29
	0	19.11	—

*4 mice/group were implanted with a 3-mm³ tumor fragment sc.
†Administered ip.
‡Relative tumor weight = ratio of mean tumor weight of a group at any given time and mean tumor weight for that group at initiation of treatment.
§ $T/C = \frac{\text{test animal mean tumor weight}}{\text{control animal mean tumor weight}} \times 100.$

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TABLE 5.—Effect of AT-125 against human small cell carcinoma (LX-1) xenograft in nude mice*

Dose† (mg/kg/injection)	Lowest relative tumor weight‡	T/C§ (%)
32	1.13	21
16	1.66	31
8	2.17	41
0	5.36	—

*4 mice/group were implanted with a 3-mm³ tumor fragment sc.
†Injected daily × 9 ip.
‡Relative tumor weight = ratio of mean tumor weight of a group at any given time and mean tumor weight for that group at initiation of treatment.
§ $T/C = \frac{\text{test animal mean tumor weight}}{\text{control animal mean tumor weight}} \times 100.$

TABLE 6.—Effect of AT-125 against human colon tumor xenografts in nude mice*

	Tumor					
	CX-1		CX-2		CX-3	
Schedule†	Optimal dose (mg/kg)	T/C‡ (%)	Optimal dose (mg/kg)	T/C‡ (%)	Optimal dose (mg/kg)	T/C‡ (%)
Daily × 10	NT§		16	81	8	249
q4days × 3	NT		100	76	NT	
Daily × 9	20	74	NT		NT	

*4 mice/group were implanted with a 3-mm³ tumor fragment sc.
†Administered ip.
‡$T/C = \frac{\text{test animal mean tumor weight}}{\text{control animal mean tumor weight}} \times 100.$
§NT = not tested.

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High-Dose Methylprednisolone, Vitamin A, and Vitamin C in Rats Bearing the Rapidly Growing Morris 7777 Hepatoma [1,2,3]

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SUMMARY

Male Buffalo rats bearing the rapidly growing Morris 7777 hepatoma were injected with various combinations of methylprednisolone (32 mg/100 g), vitamin A (40,000 IU/100 g), vitamin C (20 mg/100 g), and appropriate vehicle placebos every third day for four treatments. All animals were killed 4 days after the last injection. Tumor growth rate and a number of host responses were measured. Neither vitamin A nor vitamin C was associated with a significant change in tumor growth rate. Those groups of rats that received methylprednisolone showed a significant decrease in the hepatoma growth rate without significant change in body weight and voluntary food intake; however, these animals developed severe depression of lymphatic tissues and bleeding from the gastrointestinal tract. Vitamins A and C given together antagonized methylprednisolone-induced gastrointestinal bleeding. Rats administered this dose of vitamin A demonstrated a significant weight loss associated with low food intake, skin lesions, and hair loss. Animals given vitamin A and/or vitamin C developed a lymphocytosis whether or not they received methylprednisolone. Vitamin C enhanced splenic white pulp, suggesting an increase in lymphocyte production or storage. The results of the combined administration of methylprednisolone with vitamins A and C in Buffalo rats harboring large rapidly growing Morris 7777 hepatomas suggest areas of interest which warrant further study in this and other tumor systems.

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Currently, multiple-drug therapy is commonly used in clinical chemotherapy protocols. Few animal studies analyze combined host and tumor effects of several agents. The present study utilizes three common but relatively safe agents, methylprednisolone, vitamin A, and vitamin C, which may have either antagonistic or synergistic effects on tumor growth or body metabolism.

A glucocorticosteroid is often combined with two or three other cancer chemotherapeutic agents to provide effective therapy for leukemias and lymphomas (1–3) as well as hepatomas (4). The rationale for including glucocorticoids in such chemotherapy schedules is based on their selective toxic effects on human leukemic lymphocytes as compared to normal lymphocytes and their enhancement of cancer chemotherapy (5–7).

Previous studies of the mechanism of action of glucocorticoids in the rat Morris hepatoma liver and thymus provide clues to their selective effects or toxicity for some cell types (8–10). These reports suggest that the glucocorticoid hormones bind to a

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specific cytoplasmic receptor which moves into the nucleus where it then binds to specific nuclear sites. The lack of biologic responses in some cells following glucocorticoid administration has been attributed to a paucity or absence of effective specific cytoplasmic receptors and/or nuclear binding sites (8).

Glucocorticoids, though of proven value in some cancer therapy protocols, also have well-known harmful effects including peptic ulceration with gastrointestinal bleeding (11), suppression of the immune system and inflammatory response with decreasing levels of gamma globulins (12–14), increase of skin graft survival (15), leukopenia (13,16–18), and inhibition of wound healing (19). There is also development of hypertension with salt and water retention and excess potassium losses. Considering the benefits of glucocorticoid administration, as well as their harmful side effects, the present study was carried out to measure any inhibitory effects that methylprednisolone may have on the growth of the transplantable rat hepatoma and also to observe some of the side effects of this drug.

High-dose vitamin A given alone and in combination with methylprednisolone in the same rat tumor system was also examined. Recent reports suggest that high levels of vitamin A antagonize some side effects of methylprednisolone and may also have antitumor properties. Hutcher et al (11) have reported that vitamin A reduced gastric ulcer formations in methylprednisolone-treated rats and inhibited the corticosteroid-induced loss of mucoid substances in the gastrointestinal tract. Also, Hunt et al (19) showed that vitamin A prevented the retardation of wound healing produced by corticosteroids. On the other hand, Neifeld et al (15) reported that the increased survival time of rats with skin allografts, treated with methylprednisolone, was not significantly altered with vitamin A administration.

The antitumor effects of high-dose vitamin A have been reported in several animal tumor systems including the mouse mammary adenocarcinoma (20), the viral-induced Maloney murine sarcoma (21), and the rat chondrosarcoma (22). Human basal cell carcinomas have been reported to regress following the local application of vitamin A (23).

Unfortunately, high-dose vitamin A also has harmful side effects which include loss of appetite, skin and hair deterioration, reduced growth, muscular weakness, and skeletal defects (24–26) as well as an increase in liver gluconeogenesis and liver hypertrophy (27–29). The present study examines some of the deleterious effects of this drug and addresses the question of whether any harmful or antitumor effect of methylprednisolone is enhanced or inhibited by vitamin A.

Finally, this study tests the effects of vitamin C in this tumor model. High doses of this drug are relatively safe (30) but the rationale for including it in this study is based on reports that: (*a*) cancer patients often have an abnormally low blood level of vitamin C (31–33), (*b*) cancer patients treated with vitamin C demonstrate an enhanced lymphoblastic response to mitogens (34), (*c*) vitamin C may suppress neoplastic cellular proliferation and invasiveness via its enhancement of the synthesis of extracellular ground substance (32,35), and (*d*) vitamin C has shown antimutagenic and anticarcinogenic actions (36,37).

This study tested the effects of high-dose methylprednisolone, vitamin A, and vitamin C on the rat tumor system to determine their synergistic or antagonistic interactions on tumor growth and on various host responses.

MATERIALS AND METHODS

Male Buffalo rats weighing 240–260 g (Simonsens' Laboratories) were randomized into eight groups of five animals each. Each group was housed in an individual plastic cage and was fed commercial laboratory chow (Wayne Lab-Blox, Allied Mills, Inc) and water ad libitum. All rats were inoculated sc in the right flank with a 0.25-ml suspension of Morris 7777 hepatoma cells. This suspension was prepared by pressing viable tumor through a 40-mesh stainless-steel screen and suspending it in an equal volume of Hanks' solution (Difco Laboratories).

At the time of the first treatment, 21 days after tumor inoculation, two of the rats did not have a measurable tumor. Thus, the vitamin A group and the vitamin A plus C group had only four instead of five rats. Tumor size was determined every 3 days by caliper measurements and the largest tumor cross-sectional area calculated as described elsewhere (38).

The solutions for injection consisted of the following: normal saline (0.4 ml/100 g of body weight), Aquasol A placebo (0.8 ml/100 g of body weight; 5.0-ml vial of sorethytan oleate, USV Pharmaceutical Corp), methylprednisolone (32 mg/100 g of body weight; Solu-Medrol, 40 mg/ml of sterile water, The Upjohn Co), vitamin A (40,000 IU/100 g of body weight; Aquasol A Parenteral, 50,000 IU/ml, USV Pharmaceutical Corp), and vitamin C (20 mg/100 g of body weight; Cevalin, 100 mg/2 ml of sterile water, Eli Lilly and Co).

All groups received ip injections every 3 days for a total of four treatments. The various treatment

groups are as follows: (*a*) vehicle control (normal saline plus Aquasol A placebo), (*b*) vitamin A plus normal saline, (*c*) vitamin C plus Aquasol A placebo and normal saline, (*d*) vitamins A and C plus normal saline, (*e*) methylprednisolone plus Aquasol A placebo and normal saline, (*f*) methylprednisolone and vitamin A plus normal saline, (*g*) methylprednisolone and vitamin C plus normal saline, and (*h*) methylprednisolone and vitamins A and C.

Four days after the last treatment, all animals were killed by ether overdose. Measurements were made of terminal body weight (in grams) and tumor, liver, spleen, and thymus weights, and the organ weights were calculated as a percent of body weight. Hematology data included lymphocyte, monocyte, and neutrophil counts per milliliter of blood as well as hematocrit and hemoglobin levels (expressed as percent transmittance). Occult blood in the stool was determined (Hematest reagent tablets, Ames Co). After weighing, tumor, thymus, spleen, and liver tissues were fixed in 10% neutral formalin buffered in 0.1 M Sorensen's phosphate solution (pH 7.3) for histologic preparation. Five-μm thick histologic sections of the tissues were stained with Harris' hematoxylin and eosin (H & E). The percent of white pulp in histologic sections of the spleen was determined by the intercept point-counting morphometric procedure using a calibrated ocular grid (39).

Analysis of variance statistics were used to test statistical differences. After an F ratio was found to be significant, a critical difference between means was calculated to determine which of the means being compared were significantly different (40).

Means of the various groups of treated rats were also subjected to analysis of variance statistics. These grouped means included the slopes of body weight percent change from initial weight per day, the slopes of tumor growth (cross-sectional area in cm^2 per day), and the food intake (grams per five rats, last 2 days).

RESULTS

Body Weight and Food and Water Intake

Although there were no significant differences in body weight between the eight groups prior to the start of the experiment, there were significant differences in mean terminal body weight attributable to the various drug treatments (table 1). As shown in table 2, the presence of rapidly growing

TABLE 1.—Terminal body weight (g) and organ weights (% of body weight): mean ± SEM

Treatment group	No. of animals	Body weight	Liver weight	Spleen weight	Thymus weight
Vehicle control	5	296.6 ± 6.19	10.74 ± 0.25	0.733 ± 0.043	0.336 ± 0.059
Vitamin A	4	273.8 ± 14.4	10.58 ± 0.74	0.692 ± 0.032	0.286 ± 0.036
Vitamin C	5	301.6 ± 7.22	10.27 ± 0.36	0.733 ± 0.057	0.257 ± 0.023
Vitamins A and C	4	263.3 ± 4.27	9.74 ± 0.43	0.581 ± 0.011	0.180 ± 0.018
Methylprednisolone	5	276.0 ± 7.93	9.79 ± 0.46	0.471 ± 0.019	0.180 ± 0.018
Methylprednisolone plus vitamin A	5	258.8 ± 12.90	8.77 ± 0.41	0.555 ± 0.093	0.198 ± 0.032
Methylprednisolone plus vitamin C	5	278.2 ± 3.90	9.06 ± 0.33	0.560 ± 0.053	0.187 ± 0.026
Methylprednisolone plus vitamins A and C	5	254.6 ± 10.80	9.14 ± 0.48	0.442 ± 0.031	0.170 ± 0.031
Statistical analysis of variance					
F value		8.604	2.4794	5.0741	3.3821
P value		< 0.001	< 0.05	< 0.001	< 0.005
CDBM* (0.05)		26.16*	1.255*	0.1470*	0.0968*
(0.01)		35.12	1.685	0.1973	0.1298
(0.001)		46.40	2.226	0.2606	0.1716

*Critical difference between means: means in each column which differ by these values are significantly different at the indicated level of probability (in parentheses).

TABLE 2.—Body and tumor size changes and food and water intake: mean ± SEM

Treatment group	Slope of body weight (% change from initial weight per day)	Slope of tumor growth (cross-sectional area in cm^2 per day)	Food intake (g/5 rats/ last 2 days)	Water intake (ml/5 rats/ last 2 days)
Vehicle control	–0.603 ± 0.082	0.468 ± 0.155	256	650
Vitamin A	–1.171 ± 0.177	0.284 ± 0.105	118	350
Vitamin C	–0.557 ± 0.109	0.354 ± 0.093	226	650
Vitamins A and C	–1.496 ± 0.097	0.408 ± 0.070	94	330
Methylprednisolone	–1.046 ± 0.111	0.165 ± 0.017	256	700
Methylprednisolone plus vitamin A	–1.757 ± 0.222	0.130 ± 0.014	99	415
Methylprednisolone plus vitamin C	–1.263 ± 0.050	0.255 ± 0.120	244	735
Methylprednisolone plus vitamins A and C	–1.795 ± 0.255	0.164 ± 0.054	117	470

*Statistical analysis of the pooled means of the treatment groups**

Groups compared	Column 1	Column 2	Comparison of column 1 with column 2: Student's t test value	Comparison of column 1 with column 2: *P* value
	Slope of tumor growth			
A	Groups with methylprednisolone (0.178 ± 0.027)	Groups without methylprednisolone (0.379 ± 0.039)	4.23	< 0.01
B	Groups with vitamin A (0.247 ± 0.063)	Groups without vitamin A (0.310 ± 0.065)	0.70	NS†
C	Groups with vitamin C (0.295 ± 0.054)	Groups without vitamin C (0.261 ± 0.076)	0.36	NS
	Slope of body weight change			
A	–1.465 ± 0.185	–0.957 ± 0.228	1.73	NS
B	–1.555 ± 0.144	–0.867 ± 0.172	3.06	< 0.02
C	–1.278 ± 0.264	–1.144 ± 0.238	0.38	NS
	Food intake			
A	179.0 ± 41.2	173.5 ± 39.8	0.10	NS
B	107.0 ± 6.2	245.5 ± 7.1	14.76	< 0.001
C	170.3 ± 37.9	182.3 ± 42.8	0.21	NS

*4 group values; 5 rats in each group used for each mean.
†NS = not significant.

hepatoma was associated with wasting of body weight during the 13-day course of the experiment. Groups of rats that received vitamin A either alone or in combination with vitamin C and/or methylprednisolone had a significantly greater weight loss which was clearly correlated with a significant decrease in food and water intake. Although rats treated with methylprednisolone had a lower mean terminal body weight than the vehicle control group (table 1), the difference was not significant, nor did the food and water intake appear to be significantly different in these two groups (table 2). High-dose vitamin C changed neither the rate of body weight loss nor the food and water intake when compared to the vehicle controls (tables 1 and 2).

Tumor Growth

Table 2 summarizes tumor growth rates in each group. A statistical analysis of the pooled means of those groups of rats receiving methylprednisolone alone and in combination with other agents showed significantly ($P < 0.01$) slower mean tumor growth rates compared to the groups of rats not treated with methylprednisolone. Vitamins A and C failed to demonstrate a significant influence on the tumor

growth rate as shown in the statistical analysis of pooled means (table 2).

Organ Analysis, Hematology, and Other Observations

Rats treated with methylprednisolone had a somewhat lower mean liver weight. However, addition of vitamin A and/or vitamin C to methylprednisolone further diminished liver weight to a significantly lower level than the liver weights of the vehicle controls (table 1). Although both vitamins A and C suppressed liver weight somewhat, the differences were not significant until methylprednisolone was added. Histologic examination of H & E-stained liver sections revealed no signs of liver toxicity, fatty infiltration, or other liver pathology in any of the groups.

Mean spleen and thymus weights were both significantly depressed by methylprednisolone treatment (table 1). Although neither vitamin A nor vitamin C given alone caused a significant lowering of spleen or thymus weight, given together they did significantly lower the weights of both organs (table 1). Histologic examination of the thymus from rats receiving methylprednisolone revealed a consistent striking loss of thymocytes, particularly in the cortical region. Methylprednisolone-treated rats that also received vitamin A and/or vitamin C still showed the loss of cells in the cortical areas. The distinction between thymic cortex and medulla was obliterated in all of the rats receiving methylprednisolone. Neither vitamin A nor vitamin C without methylprednisolone showed a notable loss of cortical cells or loss of distinction between cortex and medulla, even though given together there was a significant lowering of mean thymus weight.

As seen in table 1, administration of methylprednisolone was associated with a significant lowering of spleen weight when compared to controls. Histologic sections of spleen were subjected to a point-counting morphometric analysis to determine the percent of white pulp. A summary of these results is shown in table 3. Groups receiving methylprednisolone had the lowest percent of white pulp. Rats given vitamin C and/or vitamin A had the highest percentage of white pulp. Vitamin C, in particular, seemed to increase the percentage of splenic white pulp and also to antagonize the suppressive effects of methylprednisolone on white pulp. The size of splenic nodules with prominent pale-staining germinal centers seemed largest in the groups of rats receiving vitamin C, alone or together with vitamin A, when compared to all other groups.

No significant differences were noted in the monocyte and neutrophil blood cell counts or in the

TABLE 3.—Hematology data* and morphometric analysis of the % white pulp in the spleen: mean ± SEM

Treatment group (4 or 5 animals in each group)	% white pulp	Lymphocytes/ ml of blood
Vehicle control	12.82 ± 1.06	5051 ± 1034
Vitamin C	17.70 ± 2.50	9250 ± 1631
Vitamin A	14.93 ± 2.40	8071 ± 1 84
Vitamins A and C	18.00 ± 2.91	8582 ± 1337
Methylprednisolone plus vitamin C	11.15 ± 2.33	3039 ± 656
Methylprednisolone plus vitamin A	7.68 ± 0.93	6463 ± 1078
Methylprednisolone plus vitamins A and C	12.45 ± 0.72	3081 ± 869
Methylprednisolone	6.86 ± 1.12	1875 ± 473
Statistical analysis		
F value	4.914†	6.928†
CDBM‡ (0.05)	6.58‡	3096‡
(0.01)	7.54	4156
(0.001)	10.29	5490

*Analysis of variance of monocyte and neutrophil counts, as well as the hematocrit and hemoglobin levels, showed no significant differences in the different treatment groups.

†Significant *F* value ($P < 0.001$).

‡Critical difference between means: means in each column which differ by these values are significantly different at the indicated level of probability (in parentheses).

hematocrit or hemoglobin levels. On the other hand, some striking and significant differences were found in the lymphocyte counts (table 3). Methylprednisolone administration resulted in lymphopenia, as expected. Vitamin C given alone or together with vitamin A increased the lymphocyte count significantly above that of the vehicle control rats. Both vitamins C and A antagonized the lymphopenia caused by methylprednisolone. Indeed, the antagonistic effect of vitamin A on methylprednisolone-induced lymphopenia was most significant ($P < 0.01$).

Tests for occult blood in the stool were positive in all methylprednisolone-treated rats except those that also received both vitamins A and C. None of the other groups showed a positive test for occult blood in the stool. This indicates that vitamins A and C together antagonized the gastrointestinal bleeding associated with methylprednisolone.

Most of the rats treated with vitamin A showed a loss of hair on their lower abdomen, as well as crusting of the palpebrae. Neither vitamin C nor methylprednisolone appeared to antagonize these effects. None of the rats not treated with vitamin A showed skin or hair changes.

DISCUSSION

The growth rate of the Morris 7777 hepatoma was decreased by methylprednisolone and was not significantly influenced by vitamins A and C as shown in the statistical analysis of pooled means (table 2). Because the food intake and body weights of methylprednisolone-treated rats were not significantly suppressed, it would appear that the difference in tumor growth rate with methylprednisolone could be attributed to an action of the drug on the tumor rather than malnutrition. That short-term malnutrition does not invariably result in slowing tumor growth is supported by the finding that high-dose vitamin A-fed rats did show a significant decrease in both food intake and body weight without a concomitant significant slowing of tumor growth rate. Thus, during the 13-day period of this experiment, food intake was not as critical to tumor growth rate as was methylprednisolone.

That this specific tumor was not more responsive to methylprednisolone may be accounted for by a low receptor level. The number of methylprednisolone receptors in this particular tumor is unknown. The failure of vitamin A or vitamin C to significantly retard the growth rate of this tumor is also unexplained. Possibly, this hepatoma might respond to other vitamin A derivatives or analogs. Also, other tumors may respond to one or both of these vitamins.

Treatment with high doses of either methylprednisolone or vitamin A given alone resulted in the expected harmful side effects, while short-term high-dose vitamin C by itself was not associated with any noticeably significant harmful effects. However, the observed significant lymphocytosis and increase in white pulp in the spleens of hepatoma-bearing rats enhanced by vitamin C confirm previous reports of a similar lymphoblastic response in cancer patients (34).

Synergistic and antagonistic drug responses were observed in this study. For example, vitamins A and C given together reduced occult gastrointestinal bleeding associated with methylprednisolone in tumor-bearing rats. Also, vitamin A antagonized the significant lymphopenia produced by methylprednisolone alone. Vitamins A and C both were noted to increase the number of circulating lymphocytes in hepatoma-bearing rats. In this model no measurable synergism or antagonism of tumor growth rate was observed. The significance of some of these findings is not clear but seems worthy of further research, particularly the observations on lymphocytosis produced by vitamins A and C.

In summary, methylprednisolone was observed to suppress the growth rate of the transplantable Morris 7777 hepatoma in rats. Methylprednisolone caused occult gastrointestinal bleeding in these rats which was prevented by vitamins A and C given together. Methylprednisolone administration resulted in suppression of the lymphatic system with reduction in thymus and spleen weights and severe lymphopenia. Vitamins A and C, alone or in combination, resulted in a significant lymphocytosis in the tumor-bearing rats and also antagonized the lymphopenia secondary to methylprednisolone. Harmful side effects due to high-dose vitamin A, such as decreased body weight, hair loss, and crusting of the palpabrae, were not antagonized by vitamin C or methylprednisolone.

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Phase I Trial of a New Form of an Oral Administration of VP-16-213 [1,2]

Brief Reports and Preliminary Communications

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SUMMARY

A new oral form of VP-16-213 consisting of a hydrophilic soft gelatin capsule containing a VP-16-213 solution was tested for toxicity in 26 patients. The drug was administered at four different dose levels, 350, 500, 650, and 750 mg/m², in 5-day courses every 21–28 days. A dose-dependent effect was observed with regard to hematologic toxicity and alopecia. It is concluded that the drinking ampules of VP-16-213 can be replaced with the new oral capsules with a recommended initial dose of 100–130 mg/m² given in 5-day courses every 21–28 days.

[Cancer Treat Rep 63:485–487, 1979]

VP-16-213 is a semisynthetic derivative of podophyllotoxin which primarily exerts its effect in the late metaphase stage of mitosis (1). In experimental animal tumor systems, VP-16-213 has shown high antitumor activity in L1210 leukemia, P815 mastocytoma, and P1537 leukemia. In clinical trials, VP-16-213 has been effective in a variety of solid tumors and hematologic malignancies, including small cell anaplastic carcinoma of the lung, malignant lymphoma, acute leukemia, and cancer of the ovary (2–6).

In the first clinical trials, VP-16-213 was given iv (7,8). Later, lipophilic capsules with VP-16-213 suspension for oral use were produced and evaluated. The toxicity was found to be highly variable due to erratic absorption (9), a finding which was confirmed in absorption studies with the labeled compound.[4] Subsequently, another pharmaceutical preparation, drinking ampules containing a solution of the compound, proved to have predictable absorption (10).

Because of the unpleasant taste of the solution in the drinking ampules, a new hydrophilic soft gelatin capsule containing a VP-16-213 solution was developed. An investigation of the clinical toxicity of these VP-16-213 capsules is reported here.

MATERIALS AND METHODS

VP-16-213 was supplied by Sandoz Ltd in 100-mg capsules for oral use. The dose levels tested were 350, 500, 650, and 750 mg/m² distributed equally over 5 subsequent days and repeated at intervals of 21–28 days. The planned length of treatment was at least two 5-day courses or until progression of the disease.

Minimal hematologic requirements for inclusion in the study were a platelet count of $> 125{,}000/\mu l$ and a wbc count of $> 3500/\mu l$. Hepatic biochemical tests and serum creatinine levels had to be normal unless the pathologic values were due to the neoplastic disease. The above-mentioned laboratory examinations were repeated every third week, and hemoglobin levels and wbc and platelet counts were checked every week during the first 3 weeks.

The criteria for the evaluation of the tumor response were as described in previous publications on this compound (2,7,9,10).

RESULTS

Of 30 patients admitted to the study, 26 were evaluable and four were disqualified: one was lost early to followup, one received concomitant irradiation within the first 28 days, one did not take the capsule because of severe disease-related vomiting, and one died within 15 days of the start of treatment from his malignant disease. Among the 26 evaluable patients (16 males and ten females) (median age, 56 years; range, 38–72 years), 20 had received prior therapy (irradiation or extensive chemotherapy) and six were previously untreated.

Eight patients had cancer of the lung, six had

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[4]Beveridge T, Kalberer F, and Nüesch E. Internal Report: Bioavailability study with ³H-VP-16-213. Basel, Sandoz Ltd, 1974.

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cancer of the breast, and four had malignant lymphoma. In two patients the primary origin of the malignant disease was unknown. The diagnoses of the remaining eight patients were cancers of the testis, penis, cutis, colon, and uterus.

The number of evaluable patients treated at the different dose levels included five patients at 350 mg/m^2, seven at 500 mg/m^2, 11 at 650 mg/m^2, and three at 750 mg/m^2.

Among the 26 evaluable patients three received only one evaluable course of treatment. Two of these developed severe hematologic toxicity at a dose level of 750 mg/m^2, prohibiting further treatment at this dosage, and the third received concomitant irradiation during his second course, excluding him from the evaluation. The number of courses of treatment given in the remaining 23 patients were as follows: two in nine patients, three in seven patients, four in four patients, and ten, 13+, and 14+ in three patients respectively.

Clinical Tolerance

The most consistent side effects were hematologic and gastrointestinal toxicity and alopecia. Table 1 shows the frequency and severity of hematologic toxicity. With increasing doses of VP-16-213 the frequency and severity of hematologic toxicity increased. At the 350-mg/m^2 dose level one of five patients had toxic effects as compared with five of seven patients receiving 500 mg/m^2 and ten of 11 patients receiving 650 mg/m^2. Three patients were entered at the 750-mg/m^2 level. Entry of additional patients at that level was stopped when it was observed that this dose resulted in severe anemia and severe life-threatening granulocytopenia, with the occurrence of sepsis necessitating intensive antibiotic treatment and transfusions in two of three patients.

Leukopenia seemed to be the dose-limiting factor, since the hematologic toxicity was most pronounced with regard to the wbcs and only moderate to minimal with regard to the rbcs and platelets.

The nadir of leukopenia and anemia usually occurred on Days 10–21, with return to normal on Days 21–28. Because of the severity of hematologic toxicity the interval between the 5-day courses had to be increased from 21 to 28 days in eight patients and to 35 days in one patient.

As with the hematologic toxicity, the degree of alopecia increased with increasing dose levels. Seven patients developed moderate to severe alopecia, and in four patients, all receiving VP-16-213 at a dose level of 650 mg/m^2, complete baldness occurred.

With regard to gastrointestinal toxicity, which was not dose-dependent, mild to moderate nausea (13 patients) and vomiting (six patients) were the most frequent complaints.

Antitumor Activity

Regression of > 50% of measurable tumor was seen in three of 26 patients. One patient with diffuse histiocytic lymphoma received courses at the 350-mg/m^2 level with the response occurring on Day 21 and lasting 32 days. The second patient, with metastatic cancer of the breast, received VP-16-213 at a dose of 650 mg/m^2, and regression in the cutaneous metastases occurred on Day 42 and lasted 62 days. The third patient, with mixed nodular and diffuse lymphoma, obtained a partial response at a dose of 500 mg/m^2, lasting for 273+ days. One patient had improvement with < 50% decrease in tumor size, while eight patients had no change in tumor size during treatment. The remaining patients all had progression of their disease.

DISCUSSION

Pharmacokinetic investigations with ^{3}H-labeled

TABLE 1.—Hematologic toxicity secondary to VP-16-213

Total dose given in a 5-day series (mg/m^2)	No. of patients treated	No. of patients with toxic effect/No. treated											
		Anemia				Leukopenia				Thrombocytopenia			
		I	II	III	IV	I	II	III	IV	I	II	III	IV
		(grade)*				(grade)*				(grade)*			
350	5	0	0	0	0	0	1/5	0	0	0	0	0	0
500	7	1/7	0	2/7	0	4/7	0	0	1/7	1/7	0	0	0
650	11	2/11	0	2/11	0	2/11	4/11	4/11	0	2/11	0	1/11	0
750	3	0	0	2/3	0	0	0	0	2/3	0	0	0	0

**Anemia:* I = 6.2–7.0 mmols/liter (10.0–12.0 g/100 ml); II = 5.6–6.2 mmols/liter (9.0–10.0 g/100 ml); III = 4.3–5.6 mmols/liter (7.0–9.0 g/100 ml); IV = < 4.3 mmols/liter (< 7.0 g/100 ml). *Leukopenia:* I = 2800–3500 cells/μl); II = 2000–2800 cells/μl; III = 1000–2000 cells/μl; IV = < 1000 cells/μl. *Thrombocytopenia:* I = 85,000–125,000 platelets/μl; II = 50,000–85,000 platelets/μl; III = 20,000–50,000 platelets/μl; IV = < 20,000 platelets/μl.

VP-16-213 have shown that both the drinking ampules and the new hydrophilic capsule are well-absorbed after oral intake at about 50% of the dose given.[5] The clinical results obtained in this study with the hydrophilic soft gelatin capsule containing VP-16-213 solution demonstrate a dose-dependent hematologic toxicity, which is in accordance with a predictable and constant absorption. Among the hematologic parameters, leukopenia appears to be the dose-limiting factor, but a toxic effect on the production of rbcs and platelets was also noted.

The antitumor activity in this study was very moderate, but most patients had widespread disease and/or had received extensive prior treatment, including combination chemotherapy and irradiation. It is noteworthy, however, that an objective response was observed in two patients with malignant lymphoma and in one patient with cancer of the breast.

Based upon these results and the pharmacokinetic data, the oral solution (drinking ampules) can be replaced with the new oral capsules, thus avoiding the unpleasant taste of the solution. An initial dose of 100–130 mg/m^2 daily given in 5-day courses every 21–28 days is recommended as a safe dosage inducing definite but tolerable toxicity.

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[5]*See* footnote 4.

Methotrexate, Bleomycin, and High-Dose *cis*-Dichlorodiammineplatinum(II) in the Treatment of Advanced Epidermoid Carcinoma of the Head and Neck [1,2]

Brief Reports and Preliminary Communications

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The outlook for patients with recurrent or metastatic squamous cell carcinoma of the head and neck is bleak. Responses have been seen with several chemotherapeutic agents but are generally of short duration (1,2). Methotrexate or bleomycin represents standard therapy with objective response rates of between 20% and 50% (1,2).

cis-Dichlorodiammineplatinum(II) (CDDP), a transitional metal compound, has activity in head and neck cancer (3) and was well-tolerated when given in high doses with hydration and mannitol diuresis (4,5). High-dose CDDP alone (4,5) or in combination with bleomycin (6,7) has produced response rates of approximately 36%. The current protocol, a pilot study of the Eastern Cooperative Oncology Group (ECOG), examines the efficacy and potential toxicity of high-dose CDDP used in combination with methotrexate and bleomycin.

PATIENT SELECTION

Eligibility requirements for this study included histologic confirmation of stage III or IV squamous cell carcinoma of the head and neck, measurable disease, and adequate renal function (creatinine clearance < 70 ml/minute) and cardiac function. Patients with an ECOG performance status of 4 were excluded. Nineteen patients, 18 males and one female, ranging in age from 45 to 74 years were studied. Fifteen patients had received prior radiotherapy, seven had received prior surgery, and two had received prior therapy with methotrexate and/or bleomycin. Two patients had received no prior treatment. Primary tumor sites and prior therapy are shown in table 1. Eighteen patients were evaluable for response.

TREATMENT

Bleomycin and methotrexate were given on Day 1 at doses of 15 and 20 mg/m^2 iv respectively. CDDP was administered to hospital patients on Day 2 with mannitol diuresis using a method modified from Hayes et al (4). After pre-hydration with 2 liters of 5% dextrose in 1/2 normal saline over 4 hours and after an iv bolus of 12.5 g of mannitol, CDDP was infused at a dose of 120 mg/m^2 iv over 30 minutes with 400 ml/hour of 5% dextrose in 1/2 normal saline with 10 g/hour of mannitol for 6 hours. For the next 18 hours, patients received 200 ml/hour of 5% dextrose in 1/2 normal saline. Hourly inputs and outputs were closely monitored. Electrolytes were obtained at 6 and 24 hours and potassium was supplemented when necessary. The vigorous hydration was used to prevent both the intrinsic nephrotoxicity of high-dose CDDP and the possible additive nephrotoxic potential of recently administered methotrexate. Bleomycin and methotrexate were then administered in the outpatient clinic at doses of 15 and 40 mg/m^2 iv respectively every 2 weeks for four doses and the entire three-drug combination was repeated at 10 weeks. No patient received more than two courses of the three-drug combination.

STUDY METHODS

Each patient had a complete history and physical examination with tumor measurements prior to

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TABLE 1.—Patient characteristics

Patient No.	Prior treatment*	Tumor site	Response	Duration of response (mos)	Survival (mos)
1	RT	Hypopharynx	Disease progression	—	2
2	None	Pyriform sinus	PR	5	9
3	RT	Pharynx	PR	3	7
4	S, RT, C	Tonsil	PR	4	7
5	S, RT	Glottis	CR	14+	—
6	S, RT	Alveolar ridge	PR	3	8
7	S, RT	Hypopharynx	PR	3	5
8	RT, C	Tongue	PR	5	6
9	RT	Alveolar ridge	PR	2	3
10	RT	Pyriform sinus	PR	6	—
11	None	Esophagus	PR	7+	—
12	S	Lip	PR	4	5
13	S, RT	Unknown	Not evaluable	—	< 1
14	None	Unknown	Stable disease	1	2
15	RT	Pharynx	CR	3	8
16	S, RT	Tonsil	CR	3	—
17	RT	Tongue	Disease progression	—	—
18	RT	Tongue	Stable disease	1	3
19	RT	Larynx	PR	4	—

*RT = radiotherapy; S = surgery; C = chemotherapy.

treatment. Baseline creatinine clearance, cbc, platelet count, uric acid, liver function studies, chest roentgenogram, and audiogram were obtained. The patients were re-evaluated with a clinical examination, cbc, platelet count, and serum creatinine at least every 2 weeks prior to each course of CDDP and when clinically indicated.

Standard ECOG objective response criteria were used. Response was defined as follows: *complete (CR)*—the disappearance of all palpable or radiologic lesions for > 1 month; *partial (PR)*—a ≥ 50% tumor regression (product of two diameters) for > 1 month determined by palpation or roentgenogram; *stable disease*—no change in any indicator lesion; and *disease progression*—an increase in indicator lesions or the appearance of new tumors.

There were 14 responders among the 19 patients; these included three CRs and 11 PRs. One patient had stable disease, two had disease progression, and one died 7 days after CDDP administration. An autopsy failed to incriminate the chemotherapy. The CR rate was 16% and the PR rate was 58% (total objective response rate, 74%) for all 19 patients. The mean and median durations of response were both 4 months. Two patients are still in remission at 7+ and 14+ months. Nine of the 14 responders have died with a median survival of 6 months. The median survival of the nonresponders was 2 months.

TOXICITY

Toxicity was significant. One drug-related death occurred secondary to leukopenia with sepsis. Two life-threatening complications occurred; one patient had severe nephrotoxicity and one patient had respiratory arrest resulting from severe mucositis.

Sixteen of the 19 patients developed nausea and vomiting which was considered severe in four patients, moderate in eight, and mild in four. Two patients had no nausea or vomiting. Three patients developed mucositis which was severe in two. The third patient had mucosal sloughing with a respiratory arrest although he subsequently recovered.

Seven of the 19 patients developed leukopenia. Five episodes were mild with nadirs of 1900–3800 cells/mm^3 at Days 15–18. One of two episodes of severe leukopenia resulted in death from sepsis. Nadirs were at Days 16 and 30 with wbc counts of 500 and 900/mm^3 respectively. Thrombocytopenia was not observed.

Eight of the 19 patients developed nephrotoxicity. Seven episodes were mild and transient with serum creatinines ranging from 1.7 to 2.8 mg/dl. Creatinine clearances fell from 40% to 70% below baseline level. Renal dysfunction usually began 48 hours after the CDDP infusion and recovery was complete by Day 15, with creatinine clearances returning to within 30% of the baseline level. One patient developed severe, prolonged nephrotoxicity with a serum creatinine of 8 mg/dl and a creatinine clearance of 10 ml/minute. Renal function gradually improved, but the patient died from advancing cancer at 2 months. Autopsy showed that one kidney had papillitis consistent with dehydration. Other toxic effects included one episode each of acute gout 24

hours after CDDP administration, hyponatremia consistent with inappropriate antidiuretic hormone secretion, and pulmonary fibrosis in a patient who received a dose of 165 mg of bleomycin. One patient developed hearing loss.

CONCLUSION

This schedule of high-dose CDDP, methotrexate, and bleomycin was an effective combination for advanced squamous cell carcinoma of the head and neck but the remissions (median duration, 4 months) were short. No relation between response and primary site, metastatic sites of involvement, performance status, or prior treatment could be established due to the small number of evaluable patients. The median survival of responders and nonresponders was 6 and 2 months respectively. Toxicity was moderate to severe, with one death and two life-threatening complications.

We feel that this particular combination is too toxic for general use but the data are presented to encourage dose and schedule modifications which would result in treatment programs that are better tolerated and produce longer remissions.

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Combined DTIC and *cis*-Dichlorodiammineplatinum(II) Therapy for Patients With Disseminated Melanoma: A Northern California Oncology Group Study [1,2]

Brief Reports and Preliminary Communications

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Chemotherapy for patients with disseminated melanoma is generally unsatisfactory. DTIC, the standard single agent used for treating these patients, has yielded an objective response rate of between 15% and 30% (1–5). A disproportionately large number of these responses have been reported in female patients with nodal or dermal disease (1,3,5). Attempts to combine DTIC with other active single agents (such as one of the nitrosoureas, hydroxyurea, actinomycin D, or a vinca alkaloid compound) have not conclusively demonstrated improved response or survival rates (3–7). It has been suggested that DTIC in combination with these agents may be more beneficial than single-agent therapy in the treatment of patients with visceral disease (1).

cis-Dichlorodiammineplatinum(II) (CDDP) is a relatively new chemotherapeutic agent that has demonstrated activity when used to treat genitourinary and squamous epithelial tumors (8). A preliminary report of its efficacy in the treatment of melanoma patients resulted from the phase I–II study that took place at Roswell Park Memorial Institute (Buffalo, NY). Three objective regressions were noted among 11 patients with metastatic melanoma treated with this drug (9).

When the present study was conceived, no studies had been published that reported the use of CDDP in combination with other chemotherapeutic agents for the treatment of malignant melanoma. Subsequently, Arthur D. Little, Inc (Cambridge, Mass) conducted a test of the simultaneous use of DTIC and CDDP on the B16 melanoma animal model of the National Cancer Institute. Results of that test showed additive benefit from the combination of these two drugs and prompted the present study.[5]

MATERIALS AND METHODS

Eighteen patients with metastatic, biopsy-proven melanoma were entered in this study. Two of these patients were female, 16 were male, and all were between 7 and 72 years of age (median, 45 years; mean, 43 years). Only two patients had received previous chemotherapy; only two had no visceral disease. Table 1 lists the sites of disease in these patients.

Chemotherapy consisted of both DTIC and CDDP given on the first day of treatment and repeated at 28-day intervals. The initial dosage of DTIC was 600 mg/m^2 administered as an iv infusion over a 30–60-minute period. The initial dosage of CDDP was 75 mg/m^2 as an iv infusion administered over a 6-hour period. Simultaneous and subsequent diuresis was induced with saline and mannitol. Doses of both DTIC and CDDP were increased for each course by 20% unless toxicity or progression (as described below) occurred. From one to seven courses of the drugs were administered; the average CDDP dose was 86 mg/m^2, and the average DTIC dose was 677 mg/m^2. No dose reduction was required because of toxicity.

Responses were defined as follows: *complete regression (CR)*—a disappearance of all evidence of disease for at least 4 weeks; *partial regression (PR)*—a decrease of > 50% in the sum of the products of the perpendicular dimensions of all measurable lesions for at least 8 weeks, no increase in any

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[5]Mahel JA. Personal communication. Experimental Therapeutics Section, Arthur D. Little, Inc, Apr 27, 1977.

TABLE 1.—Sites of disease and results of DTIC plus CDDP therapy

Patient No.	Sex	Sites of disease*	Response	Duration of response (days)	Survival (days)
1	M	Lung and sinus	Stable disease	126	221
2	M	Liver, GI, lung, and GU	Stable disease	122	168
3	M	GI, GU, nodes, and mesentery	Progression	—	152
4	F	Mesentery, nodes, and subcutaneous	PR	77	291
5	M	Lung	CR	113	210
6	M	Pleura and mediastinum	Progression	—	98
7	F	Liver, lung, bone, and subcutaneous	Progression	—	83
8	M	GI and nodes	Stable disease	170	210
9	M	Mediastinum, nodes, and subcutaneous	Progression	—	63
10	M	Bone	Stable disease	78	118
11	M	Liver, lung, mesentery, and pleura	Progression	—	54
12	M	Liver and mediastinum	Progression	—	58
13	M	Brain, subcutaneous, and mesentery	PR	67	172+
14	M	GI and marrow	Progression	—	94
15	M	Lung and skin	PR	117+	144+
16	M	Mesentery and pancreas	PR	35+	91+
17	M	Lung	Stable disease	56	83+
18	M	Lung, skin, and nodes	CR	33	89+

*GI = gastrointestinal; GU = genitourinary.

other indicator lesion, and an absence of new areas of disease; *stable disease*—a decrease of $< 50\%$ or an increase of $< 25\%$ in the sum of the products of the perpendicular dimensions of all measurable lesions for at least 4 weeks; and *progression*—an increase of $> 25\%$ in the sum of the products of the perpendicular dimensions of all measurable lesions or the appearance of new metastases, including lesions in the liver, lung, or brain when these organs were previously uninvolved.

RESULTS

All 18 patients could be evaluated for toxicity and response. Table 1 lists the patients' sites of disease, response to treatment, duration of response, and survival time. Two patients (11%) had a CR of tumor with a median duration of 73 days (median survival, at least 150 days). Four patients (22%) had a PR of evaluated disease with a median duration of at least 72 days (median survival, at least 158 days). All responses were noted within the first cycle (28 days) of therapy. Five patients (28%) had disease stabilization with a median duration of 122 days (median survival, at least 168 days). Seven patients (39%) had disease progression (median survival, 83 days). No objective regressions were noted in patients with hepatic, enteric, or bony disease.

In general, the toxic effects of this regimen were minimal. With one exception, all patients received at least two courses of chemotherapy and one escalated dose of drug. All patients experienced nausea and vomiting. Leukopenia was present but minimal (median nadir wbc count, 4900/μl). The major toxic effect was reproducible thrombocytopenia, which occurred 2 weeks after the drugs were administered (median nadir platelet count, 193,000/μl); usually the abnormally low platelet counts rebounded to above baseline values by the third week. The platelet count was $< 50{,}000/\mu$l on only one occasion.

Nephrotoxicity was also insignificant. Serum creatinine values were frequently elevated after each treatment, but no count was > 2.3 ml/dl, and the elevated values usually returned to normal before the next course of treatment. Serial creatinine clearances were obtained in 14 patients. There was no change in ten patients, improvement in two, and minimal deterioration in two others.

Most patients had eventual progression of disease in previous sites of known metastasis; however, one patient had an isolated "sanctuary" failure (leptomeningeal melanoma).

DISCUSSION

A combination of DTIC plus CDDP has been previously employed in the treatment of patients with disseminated melanoma. Ahmann et al (10) reported three responses among 30 patients (two in visceral- and one in nonvisceral-dominant disease). The major difference between their study and ours is that we employed twice the mean dose of CDDP (86 mg/m^2 vs 40 mg/m^2). Whether this increased dose is important or whether our better response rate was a chance occurrence cannot be determined.

Recognizing that six objective regressions were noted in our group of patients whose prognostic characteristics were unfavorable (the series was primarily composed of men with visceral disease), we think that our results are better than would ordinarily be expected. Furthermore, the regimen was well-tolerated by patients and required only 2 days of hospitalization each month. The lack of nephrotoxic effects confirms a recent report (11).

This study is a preliminary one, and whether this regression rate can be maintained in a larger group of patients remains to be determined. However, at this time we consider the combination of DTIC plus CDDP to be clinically promising and believe that the use of these agents in the therapy for patients with disseminated melanoma deserves further investigation.

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Weekly Administration of *cis*-Dichlorodiammineplatinum(II) in Childhood Solid Tumors: A Southwest Oncology Group Study [1,2]

Brief Reports and Preliminary Communications

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cis-Dichlorodiammineplatinum(II) (DDP) is an inorganic compound with antineoplastic efficacy in various types of adult and childhood malignancies (1–4). The Pediatric Division of the Southwest Oncology Group (SWOG) reported that the drug was effective against neuroblastoma, testicular embryonal carcinoma, and osteogenic sarcoma in children who were resistant to conventional therapy (4). It was ineffective in acute lymphocytic leukemia. The drug was given at a dose of 15–20 mg/m^2/day × 5 days by iv push and repeated every 3 weeks.

The optimal administration of DDP in man is unknown. In vitro studies have suggested that the fraction of cells killed by DDP is a direct function of duration of exposure of cancer cells to the drug (5). There is some clinical evidence that a schedule of weekly doses given over a period of 6–8 hours together with a hydration diuresis program is superior to the one used in the previous study (6). For that reason, the Pediatric Division of SWOG initiated a weekly program to study the toxicity and efficacy of DDP in children with various types of solid tumors.

METHODS

Patients with solid tumors were eligible for the study if they (*a*) were ≤ 18 years old at the time of diagnosis and ≤ 21 years old at the time of entry in the study, (*b*) had disease progression, (*c*) were resistant to conventional therapy, (*d*) had not received prior DDP, (*e*) had one or more measurable lesions not recently treated with radiation therapy, (*f*) had not received chemotherapy for 1 week previously, (*g*) did not show any significant toxic signs from previous therapy, and (*h*) had serum creatinine levels ≤ 1 mg/100 ml (if the patient was < 5 years old) and ≤ 1.2 mg/100 ml (if the patient was > 5 years old). Patients with only one functioning kidney were excluded. An informed consent was obtained according to the guidelines of each institution.

Using a weekly treatment schedule, 1 mg/kg of DDP was infused over a period of 8 hours. Iv hydration consisted of 3000 ml/m^2 of one-fourth normal saline in 5% dextrose in water and 3 mEq/kg of potassium over a period of 24 hours. Furosemide (Lasix) was given by iv push at a dose of 0.5 mg/kg 30 minutes before initiation of DDP infusion and at a dose of 1.5 mg/kg during DDP infusion. Mannitol at a dose of 250 mg/kg was added to the DDP solution. If the urine output was < 5 ml/kg/hour, the iv fluid rate was increased. If that was not effective, iv furosemide was repeated. Chlorpromazine (Thorazine) at a dose of 10 mg/m^2 iv was given 30 minutes before DDP infusion, 2 hours after, and then every 4–6 hours as necessary. Body weight, serum electrolytes, BUN, creatinine, and urinalysis were required before and 8 and 24 hours after DDP was initiated. Cbcs were done weekly. Creatinine clearance and audiometry were recommended but optional. If neutropenia (< 1000 neutrophils/μl) and/or thrombocytopenia (< 50,000 thrombocytes/μl) occurred, DDP treatment was delayed until the granulocyte count was ≥ 1500/μl and/or the platelet count was ≥ 100,000/μl. The patients were removed from the study if the serum creatinine increased by 25% or the creatinine clearance decreased by 30% of the baseline value.

Because of the nature of solid tumor responses, the effectiveness of DDP was evaluated after a minimum of two doses and a survival of > 14 days

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if the tumor progressed. If the disease was stable or improved, four weekly doses of DDP and a survival of > 28 days were required. Because of the nature of DDP toxicity, one dose of DDP and a survival of > 7 days were required for the evaluation of toxicity. The SWOG criteria for solid tumor responses were as follows: *complete remission (CR)*—complete disappearance of all measurable lesions; *partial remission (PR)*—regression of all tumors by ≥ 50%; and *improvement*—regression of all tumors by < 50% (one or more metastatic lesions may show > 50% reduction in size).

RESULTS

Thirty children were entered in this study. Two children were not evaluable because they died during the first week. Of the 28 evaluable patients, eight had Ewing's sarcoma, seven had neuroblastoma, two had osteogenic sarcoma, one had rhabdomyosarcoma, and ten had miscellaneous malignant tumors. The 28 children stayed in the study between 9 and 164 days (median, 25 days). The number of weekly DDP doses given to the patients ranged from one to 17 doses (median, three doses).

Twenty-four patients who received two weekly DDP doses and were in the study for > 14 days were evaluated for DDP efficacy. Nine of these patients were treated with four or more weekly doses. One of two patients with osteogenic sarcoma had a PR. This response occurred in a 15-year-old boy who had metastases in the right lower lobe of the lung and a pleural effusion. Three weeks after initiation of DDP, the metastases had decreased by > 50% and the pleural effusion had disappeared. This patient was removed from the study on Day 53 because of renal toxicity (see *Toxicity*). One of the five evaluable patients with neuroblastoma showed improvement. This response occurred in a 2-year-old girl who had widespread metastatic bony lesions of both femurs, both humeri, and the skull. Partial clearing of the osteolytic lesions and beginning of recalcification were observed 100 days after initiation of therapy. On Day 164, after 17 weekly doses of DDP, the bony lesions worsened again and the bone marrow was replaced by neuroblastoma cells. The other four patients with neuroblastoma had progressive disease after two or three DDP doses. One 4-year-old boy with an endodermal sinus tumor showed objective improvement. He had a 5 × 5-cm mass in the right buttock, a retroperitoneal mass, and multiple metastases in both lungs. By Day 35, all pulmonary lesions had disappeared, but the other lesions had increased and additional lymph nodes became enlarged. The patient received seven courses and was in the study for a total of 56 days.

Toxicity.—The toxicity of DDP was evaluated in 28 patients who received a total of 82 weekly doses of DDP. Renal toxicity was documented in four patients. In one patient, the serum creatinine level rose to 1.3 mg/100 ml. At the same time (Day 53) the creatinine clearance was 53 ml/minute/1.73 m^2. The creatinine clearance of three other patients decreased to values between 48 and 66 ml/minute/1.73 m^2 on Days 8, 21, and 37. Three of these four patients developed signs of disease progression at the time of abnormal creatinine clearance. Nine patients tolerated the drug without any vomiting. Vomiting occurred fewer than two times in seven patients, between three and six times in nine patients, and more than six times in three patients. Myelotoxicity was evaluated in patients who had a normal hemoglobin level, normal neutrophil count, or normal platelet count at the beginning of treatment. Fourteen patients had a hemoglobin level of ≥ 10 g/dl when treatment was initiated. None of these patients had decreased hemoglobin values during treatment. Four of 19 patients with a platelet count ≥ 100,000/μl at the beginning of treatment developed a platelet count between 32,000 and 89,000/μl during treatment. One of the 28 patients with neutrophil counts ≥ 1000/μl developed neutropenia (640 neutrophils/μl).

The hydration and diuresis program was effective. The urine output within 24 hours was sufficient. Marked diuresis was observed in the first 8 hours. Body weight and electrolytes were measured before and 8 and 24 hours after DDP initiation, for a total of 47 administrations. In comparison to the initial values, weights varied between +1 and −2.0 kg after 8 hours (median, −0.750 kg). Serum sodium values varied between +5 and −14 mEq (median, −4 mEq). Chloride values varied between +4 and −17 mEq (median, −7 mEq). Serum potassium, serum creatinine, and BUN levels remained within the normal range. After 24 hours, weight, serum sodium, and chloride levels returned to the initial values.

DISCUSSION

Weekly DDP administration combined with a hydration and diuresis program demonstrated antitumor efficacy in osteogenic sarcoma, neuroblastoma, and endodermal sinus tumor. Toxicity was moderate. The main toxic effects were gastrointestinal and, to a lesser extent, renal complications. The diuresis program produced loss of body weight and reductions in serum sodium and chloride levels 8 hours after DDP initiation, all of which were corrected within 24 hours.

Rozencweig et al have recently stated that the

TABLE 1.—DDP efficacy in solid tumors

Tumor	No. of patients			No. of responses			
	Total	I*	II*	CR	PR	Improvement	Total
Neuroblastoma	28	23	5	1	3	3	7
Rhabdomyosarcoma	7	6	1	0	0	0	0
Ewing's sarcoma	11	3	8	0	0	0	0
Osteogenic sarcoma	3	1	2	0	2	0	2
Testicular embryonal carcinoma	2	2	0	0	0	1	1
Retinoblastoma	2	2	0	0	0	0	0
Miscellaneous	18	10	8	0	0	1	1
Totals	71	47	24	1	5	5	11

*Therapeutic regimens: group I = 15 or 20 mg/m²/day × 5 days q3wks by iv push; group II = 1 mg/kg iv over 8 hrs with hydration and diuretics weekly.

optimal mode of DDP administration which produces antitumor efficacy in man is unknown (1,2). The two studies initiated by the Pediatric Division of SWOG demonstrated that a 5-day regimen (repeated every 3 weeks) and a weekly regimen with hydration and diuresis had antitumor efficacy (table 1). Both treatment regimens produced moderate renal and gastrointestinal toxicity.

Various hydration programs have been advocated to prevent nephrotoxic manifestations in patients treated with DDP, ie, high levels of iv fluids in combination with mannitol and/or furosemide (1). Recently it was reported that 50–75 mg/m² of DDP with 40 g of mannitol and iv fluid can be safely given to adults over a period of 2 hours (7). In this study we used the program recommended by Merrin (6). In an ongoing study, a marked diuresis was achieved by high levels of iv fluids and furosemide without mannitol.[8]

DDP is no longer given as a single agent, but is used in combination with other drugs. To establish the best mode of DDP administration, randomized comparison studies of combination therapy with DDP need to be designed in which the treatment arm differs only by the way in which DDP is administered. Because childhood tumors are infrequent, these programs should be developed in the more common malignant tumors of adults which are known to be sensitive to DDP.

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Controlled Clinical Studies With an Antidote Against the Urotoxicity of Oxazaphosphorines: Preliminary Results [1,2]

Brief Reports and Preliminary Communications

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SUMMARY

A randomized study in 20 patients with cancer was carried out to test the clinical efficacy of sodium-2-mercaptoethane sulfonate (ASTA D-7093; mesnum) as an agent to prevent urotoxic side effects (in particular, hemorrhagic cystitis) during cytostatic therapy with the oxazaphosphorines cyclophosphamide and ifosfamide. Eleven patients received mesnum iv and nine patients received a standard prophylaxis. The frequency of microhematuria was significantly lower in the patients receiving mesnum. A slight microhematuria was observed in one patient. With the standard prophylaxis, all nine patients receiving single-agent therapy with ifosfamide or cyclophosphamide had hematuria and three of these had macrohematuria. According to the available results, a daily mesnum dose of 60% (wt/wt) of the ifosfamide or cyclophosphamide dose is recommended. This dose should be divided into three equal fractions. The first administration should be given concurrently with the cytostatic agent and the subsequent two administrations at 4 and 8 hours after administration of the cytostatic agent. Significantly higher doses of mesnum (eg, 133% of the cyclophosphamide or ifosfamide doses) lead to gastrointestinal disorders, which are easily reversible.

[Cancer Treat Rep 63:501–505, 1979]

Chemotherapy for malignant tumors with oxazaphosphorines (cyclophosphamide or ifosfamide) is frequently limited by the drug's urotoxicity, appearing as microhematuria and macrohematuria, particularly due to hemorrhagic cystitis (1). This urotoxicity is induced by the renal elimination of aggressive metabolites (2). The following prophylactic measures are recommended (3): increased fluid intake, diuretics, alkalinization of the urine, and instillation of sulfhydryl (SH) group-containing agents (eg, *N*-acetylcysteine [4,5]) into the bladder. Trials of systemic administration of *N*-acetylcysteine and other SH-containing agents were unsuccessful, since not only the urotoxicity but also the specific antitumor activity was reduced (6).

With the aim of achieving regional detoxification, another SH compound, ie, sodium-2-mercaptoethane sulfonate (ASTA D-7093; mesnum), has been studied by Brock et al (7,8) in animal experiments. In order to achieve a better understanding of the clinical efficacy of mesnum, the following summarizes some important pharmacologic results (*see* Brock et al [9]):

1. The cytostatically effective oxazaphosphorines cyclophosphamide and ifosfamide lead to a dose-dependent purulent, necrotic cystitis in the rat, with edema of the submucosa and ulceration of the mucous membrane (fig 1). Kidneys and efferent renal tract are similarly affected.
2. If mesnum is injected iv at the same time as the oxazaphosphorine (eg, ifosfamide), the severe urotoxic changes to the kidney, efferent urinary tract, and urinary bladder can be completely avoided (optimal dose relationship of oxazaphosphorine to mesnum = 3:1 [wt/wt]). Figure 2 shows the urinary bladder of a rat treated with ifosfamide and mesnum (no difference is observed compared to untreated controls).
3. Mesnum by itself is only slightly toxic (the iv LD50 in the male rat is 2080 mg/kg).
4. The various pharmacotherapeutically relevant effects of the oxazaphosphorines (eg, the curative, leukotoxic, immunosuppressive, and lethal effects) are not influenced by even high doses of mesnum (doses tenfold higher than the required

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[2]This paper is dedicated to Prof. Dr. Hermann Druckrey, Freiburg i. Br., on the occasion of his 75th birthday.

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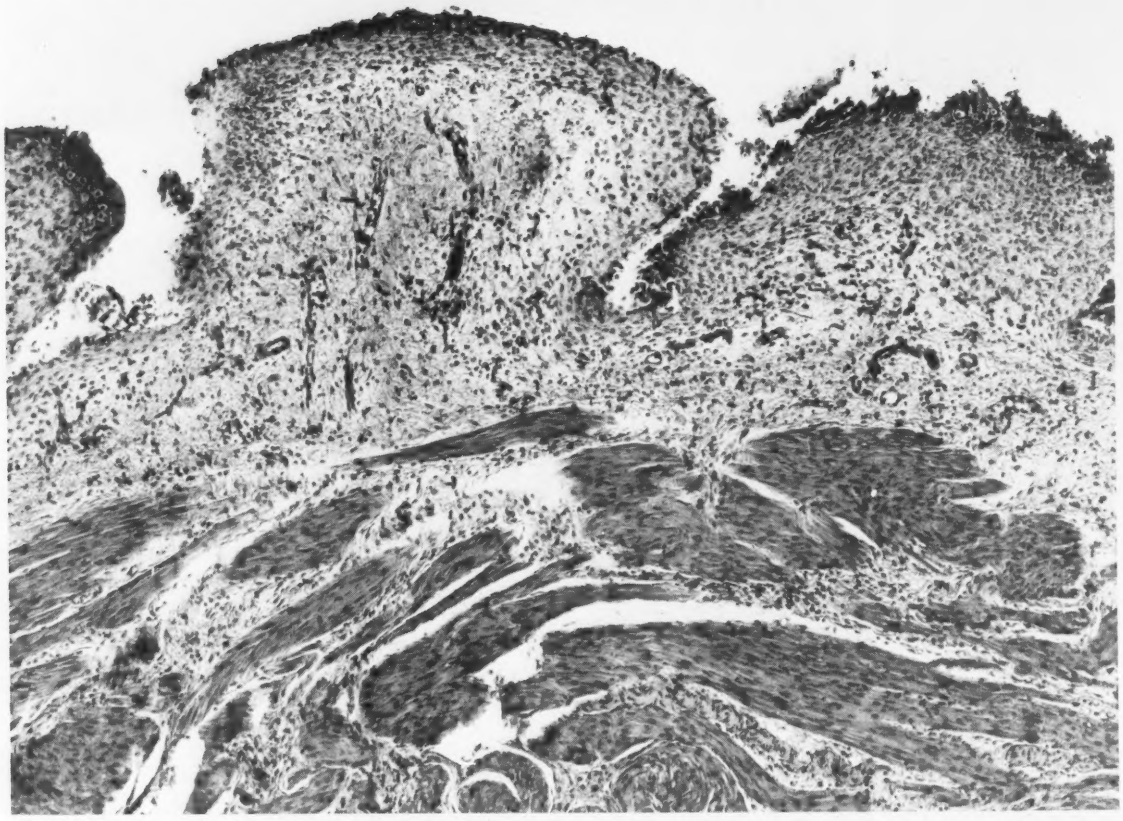

FIGURE 1.—Histology of the rat bladder 24 hrs after iv administration of 68.1 mg/kg of ifosfamide. Note detachment of the urothelium, extensive edema, and necrosis in the musculature.

prophylactic doses). The curative efficacy of the oxazaphosphorines is not reduced, as has been shown in various experimental animal tumor systems.

5. Pharmacokinetic analysis has shown that mesnum, in comparison to other SH compounds, is not taken up by tissues but is quickly and completely eliminated via the kidneys (9).
6. The specific urotoxicity of the oxazaphosphorines is most probably due to acrolein. Acrolein, derived from aldophosphamide after glomerular filtration of the primary oxazaphosphorine metabolites, combines with mesnum and is inactivated completely (10).

The tolerance limit of mesnum after iv administration in man (from results of a phase I trial) is about 70 mg/kg.[6] Nausea and vomiting were the first toxic effects. Preliminary studies by Scheef showed that iv administration of mesnum effectively prevents urotoxicity. We report here on a controlled study with this compound. It was shown at the 5% significance level that mesnum definitely reduces the incidence of hematuria and cystitis following treatment with cyclophosphamide and ifosfamide, compared to the previously used standard prophylaxis.

MATERIALS AND METHODS

Ten patients from the Janker Radiation Clinic, Bonn, W Germany, and ten patients from the Medical Unit of Cologne University Hospital, W Germany, were investigated separately. These patients were randomly allocated to either group I, with protection by mesnum, or group II, with standard prophylaxis only. At the end of the study, there

[6]Lücker PW. Unpublished results.

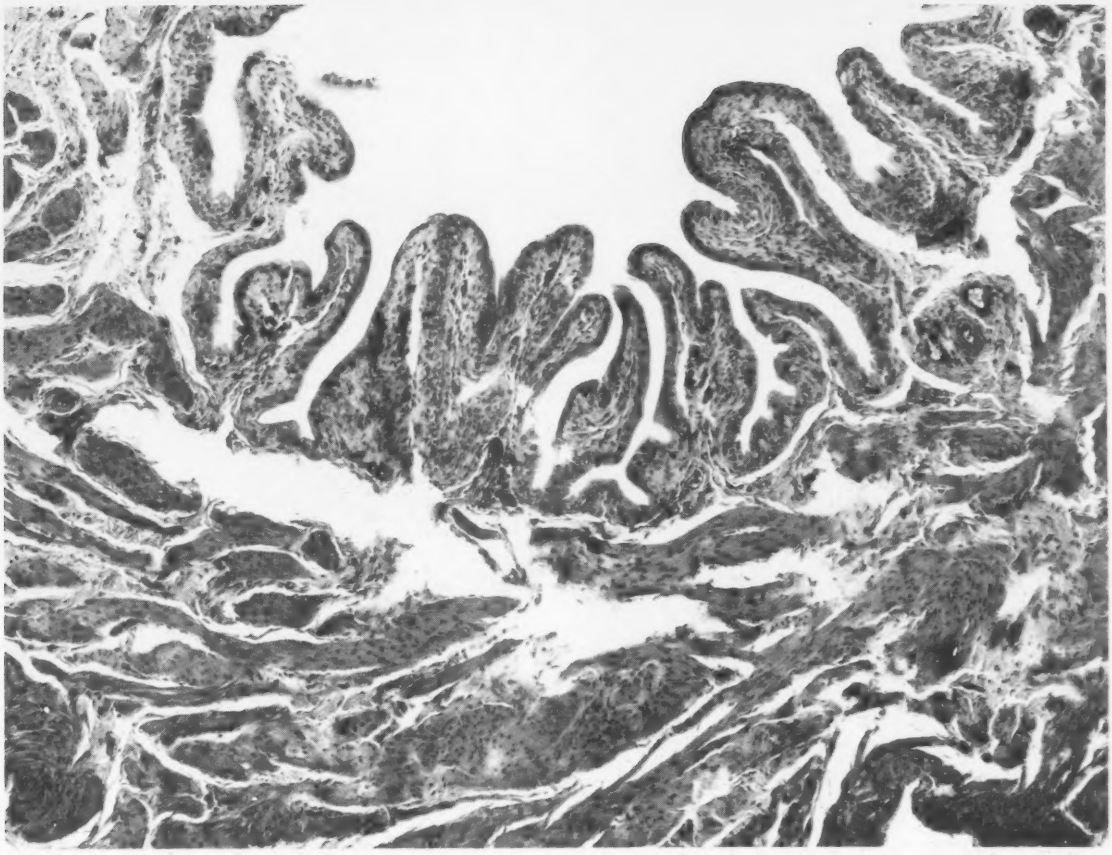

FIGURE 2.—Histology of the rat bladder 24 hrs after concurrent iv administration of 68.1 mg/kg of ifosfamide and 21.5 mg/kg of mesnum. There was no difference compared to untreated controls.

were 11 patients in group I and nine patients in group II (table 1). Additional patients were not entered in the study for ethical reasons, since a sequential analysis had already shown the positive prophylactic effect of mesnum.

Standard prophylaxis in group II included fluid intake of about 4 liters/day, administration of furosemide, and alkalinization of the urine with citric acid-citrate complexes. In three patients at the Janker Radiation Clinic, *N*-acetyl-D,L-homocysteine-thiolactone was instilled together with L-cysteine into the bladder. At the Cologne University Hospital, instillation of SH compounds was in general avoided, in order to prevent artificial bleeding in the urinary bladder due to the use of a catheter.

In group I, with protection using mesnum, the fluid intake was limited to a maximum of 2 liters/day, and diuretics were only administered when the passage of urine dropped below the critical value of 1 liter/hour in the first 6 hours following administration of the cytostatic agent.

All patients in this study received iv single-agent therapy with either ifosfamide or cyclophosphamide. In group I ifosfamide was administered at a total dose of 300 mg/kg to six patients and at a total dose of 270 mg/kg to three patients. In group II all patients received a total dose of 300 mg/kg. Ifosfamide was administered in fractionated doses. The total dose was divided into daily doses of between 60 and 100 mg/kg on 3–5 consecutive days. Cyclophosphamide was administered to patients in group I as a single dose of 48 or 50 mg/kg and to patients in group II as a single dose of 44 or 45 mg/kg.

Due to its rapid elimination, mesnum must be administered three times daily by the iv route: concurrently with the cytostatic agent, and at 4 and 8 hours thereafter. The mesnum dose is dependent on the individual cytostatic dose and is calculated

TABLE 1.—Tumor types and prophylactic measures

Cytostatic agent	Tumor type	No. of patients in— Group I (mesnum)	Group II (standard prophylaxis)
Ifosfamide	Testicular tumor	2	2
	Bronchogenic carcinoma	4	3
	Thyroid carcinoma	1	1
	Miscellaneous malignant tumors	2	1
Cyclophosphamide	Malignant lymphoma	2	2
Total		11	9

as a percentage (wt/wt) of the cytostatic dose. During the study, the mesnum dose was arbitrarily reduced from 66% + 66% + 66% of the cytostatic dose to 17% + 17% + 17% as the minimum protective dose schedule. The doses of mesnum used are shown in table 2.

The determination of erythrocytes in the morning urine during the cytostatic treatment was carried out daily. The first determination was done on the morning before the cytostatic administration; the morning urine was also examined on 4 subsequent days after completion of treatment. The occurrence of > 15 rbcs/μl of urine was considered to indicate microhematuria. Even after high doses of mesnum (80 mg/kg in humans; 500 mg/kg in rats) the drug has no hemolytic effect.

TOXICITY

Nausea was observed in seven patients in each group. Vomiting occurred in six patients in group I and in four patients in group II. Four patients in group I had diarrhea. In the mesnum group, increased vomiting and diarrhea occurred only when doses > 80 mg/kg were given. Doses of mesnum up to 60 mg/kg did not increase the cytostatic agents' gastrointestinal effects.

TABLE 2.—Dose of mesnum as a % of the cytostatic agent

Cytostatic agent	No. of patients	Mesnum % (wt/wt) of the cytostatic dose 1st dose	2nd dose	3rd dose
Ifosfamide	1	66	66	66
	4	66	33	33
	4	17	17	17
Cyclophosphamide	1	20	20	20
	1	17	17	17

RESULTS

The erythrocyte counts in the urine during treatment with ifosfamide or cyclophosphamide are summarized in table 3. Two of 11 patients in group I treated with mesnum had erythrocytes in the urine. In the case of one of these patients (cyclophosphamide treatment) a microhematuria (35 rbcs/μl of urine) was already present at the beginning of therapy. During the treatment with cyclophosphamide and mesnum no increase in microhematuria was observed. The second patient with microhematuria in group I had only 18 rbcs/μl of urine. The other nine patients did not show any signs of microhematuria or macrohematuria.

In group II, without mesnum, hematuria was observed in all nine patients; eight had > 50 rbcs/μl of urine. The differences demonstrated in table 3 between the two preventive methods are significant. Macrohematuria was observed in none of the 11 patients in group I but in three patients in group II; these latter patients had not received any instillation of SH compounds into the urinary bladder. In spite of macrohematuria, the treatment with ifosfamide could be continued in two patients after simultaneous protection with mesnum. In both cases the hemorrhage stopped within 1–2 days.

The antidote did not interfere with the antitumor efficacy of the oxazaphosphorines. The effect of treatment (either response, stable disease, or progression) can be seen in 17 of the 20 patients in table 4. In the other three patients an assessment was not possible (due to re-induction). The resulting data (table 4) show no difference between the mesnum and standard prophylaxis.

CONCLUSIONS

The protective effect of mesnum first studied in animal experiments (7,8) was confirmed by preliminary clinical trials and by these controlled randomized studies. Mesnum permits the control of urotoxicity following the use of oxazaphosphorines. The mechanism of action can be attributed to the fact that mesnum does not penetrate into the tissues,

TABLE 3.—Rbc count in urine after massive-dose administration of ifosfamide or cyclophosphamide

Prophylactic measure	No. of patients with rbcs/μl of urine ≤ 15	16–50	> 50
Group I (mesnum)	9	2	0
Group II (standard prophylaxis)	0	1	8

TABLE 4.—Results of therapy

Prophylactic measure	No. of patients with—			
	Response	Stable disease	Progression	Total
Mesnum	4	3	1	8
Standard	5	2	2	9

but is completely and rapidly eliminated in the urine. Its SH groups bind acrolein, the urotoxic metabolite of cytostatic oxazaphosphorines, in the urinary tract. The efficacy of the cytostatic therapy is in no way diminished.

A detailed paper on this new antidote will be published (9).

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Results of a Phase II Study of Maytansine in Patients With Breast Carcinoma and Melanoma [1,2]

Brief Reports and Preliminary Communications

Fernando Cabanillas,* Gerald P. Bodey, Michael A. Burgess, and Emil J Freireich [3]

SUMMARY

During the phase I study of maytansine at our institution, some activity was observed against breast carcinoma and melanoma. A phase II study was thus initiated to more thoroughly investigate the activity of this drug against these two tumors. In 33 evaluable patients with melanoma, no complete or partial responses were observed. Twenty-one evaluable patients with breast cancer were entered and only one response (partial) was seen. The toxicity was similar to that observed in the phase I study and consisted mainly of diarrhea, paresthesias, phlebitis, and flu-like symptoms. Myelosuppression was infrequent and was short-lived when it occurred.

[Cancer Treat Rep 63:507–509, 1979]

Maytansine is a recently discovered ansa macrolide drug derived from the plant *Maytenus ovatus* (1). It is active against the experimental P388 leukemia and B16 melanoma animal tumors.[4] In a phase I trial using a 3-day schedule conducted at our institute, activity was detected against breast carcinoma and melanoma (2). The dose we recommended for further clinical trials was 0.5 mg/m^2 daily × 3 every 2–3 weeks according to toxicity. The dose-limiting toxic effect of this agent was diarrhea; myelosuppression was mild or nonexistent. Consequently, it was thought that this would be an interesting compound for further clinical trials. We initiated a phase II trial in patients with breast carcinoma and melanoma in June 1977.

MATERIALS AND METHODS

Patients with advanced histologically documented carcinoma of the breast or melanoma who had failed to respond to regimens of higher priority were candidates for this study. Physical examination was performed and measurable lesions were recorded. The following pretreatment studies were performed: cbc, platelet count, urinalysis, SMA-12 profile before every cycle of treatment, bone marrow aspiration and biopsy, and chest roentgenogram. The cbc was repeated at least once a week during therapy. Other tests to evaluate the presence of measurable tumor were obtained as indicated and were repeated after two cycles of therapy in order to evaluate response to treatment.

This study was designed as a randomized comparison of two different schedules: (*a*) 0.5 mg/m^2 in 500 ml of dextrose in water over 30 minutes daily × 3 repeated in 2–3 weeks with subsequent increments of 0.1 mg/m^2 according to toxicity, or (*b*) 0.75 mg/m^2 in 500 ml of dextrose in water over 30 minutes iv weekly with 0.25-mg/m^2 increments every week according to toxicity.

Early during the study, it was found that most patients with normal liver function tests could tolerate a dose of 0.6 mg/m^2 daily × 3 and, thus, the starting dose was increased. In the weekly schedule, the starting dose was increased to 1 mg/m^2. Patients with abnormal liver function tests (SGOT or alkaline phosphatase levels increased to twice the normal levels) received a 50% dose reduction, and if the first course was well-tolerated the following dose was increased to the standard starting dose. The dose of maytansine was modified according to the severity of diarrhea. When this was severe (requiring iv fluids for treatment of dehydration or lasting > 5 days), the dose was reduced by 33%. If ileus developed, the dose was reduced by 50% after

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[2]Supported in part by contract N01-CM-57042 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[4]Helman L, Henney J, and Slavik M. Clinical brochure: maytansine. Prepared by the National Cancer Institute, Bethesda, Md, 1976.

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resolution of the obstruction. Myelosuppression was not used as a criterion for dose modification because it occurred infrequently and was almost always associated with diarrhea.

After several patients were entered in the study, the weekly schedule was discontinued at the request of the National Cancer Institute (not because of excessive toxicity). Thus, fewer patients were entered in the weekly regimen compared to the daily × 3 regimen.

RESULTS

Response of Patients With Melanoma

At the close of this study, 33 evaluable patients with melanoma had been entered in the 3-day schedule and 12 had been entered in the weekly schedule. All patients had visceral disease with or without associated nodal or skin lesions. There were no complete or partial responses. Four patients had a less than partial remission, all except one in soft tissue disease. Three of these minimal responses occurred in subcutaneous melanoma nodes which decreased in size with no evidence of a simultaneous response in visceral sites. The fourth minimal response was in a patient who had malignant ascites and pleural effusion which decreased in size with no evident response in a supraclavicular mass. Five patients were stable for 6 weeks or more and 24 failed to respond at all. Of the four less than partial responses, two occurred in the 3-day schedule and two in the weekly schedule.

Response of Patients With Breast Carcinoma

Twenty-one evaluable patients with breast carcinoma were entered. Eighteen were treated with the 3-day schedule and three with the weekly schedule. With the exception of one patient who had exclusively nodal disease, all others had visceral disease. There were no complete remissions. One patient had a partial remission (bone marrow disease) lasting 4 months and one had a less than partial remission in a supraclavicular node. Both of these patients received the 3-day schedule. Three patients had stable disease for ≥ 6 weeks and 16 failed to respond. These patients were heavily pretreated and in most cases maytansine was the third or fourth drug used.

Myelosuppressive Toxicity

Since more patients with breast carcinoma had a history of prior radiation therapy and prior exposure to myelosuppressive drugs (eg, mitomycin C) with cumulative toxicity (compared to patients with melanoma), the myelosuppressive toxicity of maytansine has been analyzed separately for patients with these two diagnoses. The myelosuppressive toxicity is shown in table 1. Myelosuppression was observed infrequently and was seen less frequently in the group that received the weekly schedule.

Gastrointestinal Toxicity

Gastrointestinal toxicity for patients with both breast carcinoma and melanoma is shown in table 2 according to the schedule of treatment. The total amount of drug in milligrams per square meter that was delivered per month is also shown in table 2. The major and most frequent toxic effect of maytansine was diarrhea, which in some cases was severe enough to require hospitalization. This problem seemed to occur less frequently with the weekly schedule. The total amount of drug delivered per month with the weekly schedule was higher, yet the toxicity was less.

Other Toxic Effects

Aside from myelosuppression and gastrointestinal toxicity, other side effects consisted of paresthesias in six patients, superficial phlebitis in four patients,

TABLE 1.—Myelosuppressive toxicity of maytansine

	3-day schedule	Weekly schedule
Melanoma		
No. of evaluable courses	33	36
No. of patients with myelosuppression*	4	1
Breast carcinoma		
No. of evaluable courses	24	8
No. of patients with myelosuppression*	7	0

*Any drop in the wbc count to < 3000/mm³ or the platelet count to < 100,000/mm³.

TABLE 2.—Gastrointestinal toxicity of 3-day vs weekly schedule compared to total maytansine dose delivered (includes both breast carcinoma and melanoma patients)

	3-day schedule	Weekly schedule
No. of evaluable courses	102	49
No. of patients with—		
Nausea/vomiting	36(35%)	17(35%)
Diarrhea	43(42%)	16(33%)
Ileus	3(3%)	1(2%)
Constipation	6(6%)	1(2%)
Dose delivered per mo (mg/m²)	3.29	4.07

flu-like symptoms in four patients, fever or chills in three patients, skin slough in one patient, and mucositis in one patient. In this study, the development of paresthesias was not related to previous vinca alkaloid administration. None of the patients who experienced this toxicity had been previously exposed to vinca alkaloids.

DISCUSSION

The response rate observed in this phase II study of maytansine has been disappointingly low. Using the schedules outlined above, maytansine cannot be considered an active drug against melanoma or breast carcinoma. However, further trials are justified in other tumors. Furthermore, it is conceivable that the schedule selected for this trial might not be the most adequate. Pharmacology studies are necessary to delineate the pharmacokinetics of this interesting compound so that a rational schedule of administration can be designed. Unfortunately, an assay sensitive enough to measure the minute levels of maytansine is not available.

The weekly schedule of administration allowed more drug to be administered with less toxicity than the 3-day schedule. This, however, did not result in a significant increase in response rate. Should this drug prove to be active against other tumors, the weekly schedule might be preferable if the drug has a sufficiently long half-life.

Because of its virtual lack of myelosuppression and overlapping toxic effects with most of the currently available drugs, this agent should be explored more extensively. Using a single-dose schedule of maytansine, the National Cancer Institute has also found very little myelosuppression (3). This compound possesses significant activity against experimental animal tumors. The type of toxicity observed in this and other studies suggests a potentially important role in combination with myelosuppressive drugs. Further exploration of its activity in other tumors and with several different schedules is necessary.

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VM-26 as a Second Drug in the Treatment of Brain Gliomas [1,2,3]

Brief Reports and Preliminary Communications

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SUMMARY

Twenty patients previously treated with surgical resection, radiation therapy, and a nitrosourea for malignant gliomas of the brain were given VM-26 as a second chemotherapeutic agent when evidence of tumor progression occurred. Thirty-five percent achieved an arrest or decrease of neurologic symptoms, with a median length of response (arrest and tumor regression) of 22 weeks.

[Cancer Treat Rep 63:511–512, 1979]

Single-agent first-drug chemotherapy for malignant brain tumors produces a response frequency of 38%–52%, depending on the agent used, while single-agent second-drug chemotherapy produces a lower response rate (15%) (1). Combination chemotherapy has not demonstrated a clear advantage in response rate compared to single-agent therapy (2–4) and may add to toxicity. VM-26, a semisynthetic podophyllotoxin derivative, produces a response rate of 50% when used as first-drug chemotherapy for malignant brain tumors (5). Three patients who had brain tumor progression while being treated with a nitrosourea were treated with VM-26 as a second single agent and two of the three responded, suggesting that VM-26 may be a useful second single-agent drug (5). In view of this observation, we initiated a trial of VM-26 as second-drug chemotherapy for patients with progressing brain gliomas.

MATERIALS AND METHODS

Twenty patients treated with a nitrosourea at The University of Nebraska Medical Center who were previously treated for malignant gliomas with subtotal resection demonstrated progression of disease and were eligible for the study. This progression was documented by computerized axial tomographic (CAT) scan in 16 patients, by nuclide brain scan in one patient, and by progressing neurologic signs (hemiparesis, aphasia, vision loss) in three patients. All had previously received radiation therapy. The median interval from the start of nitrosourea therapy to the start of VM-26 was 31 weeks and the mean was 36 weeks. At the time VM-26 therapy was administered, all patients had biopsy-proven grade III or IV gliomas. Patients had a Karnofsky functional score of 10–80 at the time of initiation of VM-26; most had a score of 70.

VM-26 was administered weekly in an outpatient setting at a dose of 100–130 mg/m^2 iv in a 5% dextrose in water infusion over a 30-minute period. If the platelet count fell to $< 100{,}000/mm^3$ or the wbc count to $< 3500/mm^3$, the drug was not administered until the blood counts returned to an acceptable range, and the dosage subsequently was reduced by one half. All patients were considered eligible for evaluation regardless of the number of doses received; one patient received only one dose, one received three doses, two received four doses, and one received five doses. Neurologic symptoms must have improved (response) or remained stable (arrest) for 8 weeks before a subjective response was said to have occurred. Objective measurements of response included length of survival and, in three patients, CAT scans of the brain.

RESULTS

Four patients (20%) had responses lasting 10, 22, 27, and 54+ weeks (one is responding at the time of this writing). Two of these responses were documented with CAT scan. The third resulted in a semi-comatose patient being able to walk, talk, and

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[2]Supported in part by grant CA-19623 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]VM-26 was supplied by the National Cancer Institute, Bethesda, Md.

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do some household chores, and the fourth had disappearance of frequent grand mal seizures with no change in medications except for VM-26. Three patients (15%) had an arrest of neurologic symptoms for 12, 9, and 29+ weeks (one is responding at the time of this writing). One of these arrests was documented by CAT scan 25 weeks after VM-26 was begun. The other two arrests were documented by stabilization of previously progressing neurologic signs without any change in medications other than VM-26. One of these patients died of a pulmonary embolus while her tumor was under control. The mean and median lengths of response and arrest are 23.2 and 22 weeks respectively. Thirteen patients (65%) demonstrated progression of neurologic symptoms despite VM-26 therapy. Median survival of the four patients who responded to VM-26 from the time of institution of the drug is 24 weeks (mean, 28 weeks). Mean survival of patients who had no change in neurologic symptoms from the time of initiation of VM-26 is 27 weeks (median, 29 weeks). Median length of survival for nonresponders after initiation of VM-26 is 9 weeks (mean, 9.1 weeks). Three of the four patients who had a remission to VM-26 had previously responded to BCNU. Of the three patients who had no progression of neurologic disease for 8 weeks or more, all previously had a response or arrest of disease while receiving BCNU. Of the 13 patients whose tumors progressed after treatment with VM-26, three failed to respond to BCNU, seven had an arrest of disease, and three had previously responded to BCNU and/or radiation therapy.

TOXICITY

Therapy was generally well-tolerated. Six patients (30%) developed platelet counts of $< 100{,}000/mm^3$, necessitating a decrease in drug dose. Three patients (15%) developed leukopenia (wbc count $< 3500/mm^3$). Alopecia, hypotension, nausea, and vomiting were not observed. One patient, however, developed lethal sepsis following a single dose of VM-26. This patient developed a wbc count nadir of $300/mm^3$ and a platelet count nadir of $6000/mm^3$. In protective isolation he developed a *Pseudomonas aeruginosa* septicemia and at autopsy was found to have an intra-abdominal abscess and acute hemorrhagic bronchopneumonia.

DISCUSSION

Second-drug single-agent chemotherapy for brain tumors has not been reported to be as effective as first-drug chemotherapy, a phenomenon not unique to this malignancy. Thus, a response rate of 35% (arrest plus response) for VM-26 as a second drug compared to a reported 50% response rate for VM-26 as a first drug (5) was somewhat surprising, although the length of response was less when VM-26 was used as a second drug. The patients receiving VM-26 reported here had a longer response (arrest plus response) to their first single-agent chemotherapy, a nitrosourea, given in conjunction with radiation in some cases (median, 36 weeks; mean, 37.2 weeks).

VM-26 as a second drug offers some continued useful time for those patients who respond. Other drugs known to be effective in the treatment of brain tumors should be evaluated as to usefulness for second-drug single-agent chemotherapy in hope of finding sequences that are more effective.

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Phase II Study of Single-Agent Therapy With Megestrol Acetate, VP-16-213, Cyclophosphamide, and Dianhydrogalactitol in Advanced Renal Cell Cancer [1]

Brief Reports and Preliminary Communications

Richard G. Hahn,* Madeline Bauer, Janet Wolter, Richard Creech, John M. Bennett, and Galen Wampler [2,3]

SUMMARY

One hundred and forty-four patients with advanced renal cell cancer were evaluated for objective response to single-agent treatment with megestrol acetate, VP-16-213, cyclophosphamide, or dianhydrogalactitol. In view of the low order of response observed, these agents do not appear to be effective in the treatment of metastatic renal cell cancer. Performance status and a relatively long interval from onset of primary symptoms to study entry were the most favorable prognostic variables affecting time to progression and survival for patients in this study.

[Cancer Treat Rep 63:513–515, 1979]

Patients with metastatic renal cell cancer represent a sizable proportion of the patients with advanced urologic cancer. Patients with stage I and II surgically treated disease have a better 5-year survival rate than patients with stage III disease, although these patients represent a minority of the overall group of patients who have resectable disease. Despite aggressive surgical treatment, the overall 5-year survival rate remains only 40%.

Progestational hormonal treatment trials for advanced disease have reported response rates ranging from 0 to 16% (1–3). A previous study of methyl-CCNU and vinblastine as single agents and in combination with medroxyprogesterone, conducted by the Eastern Cooperative Oncology Group (ECOG) (4), showed response rates comparable to those of previously reported cytotoxic chemotherapy trials in renal cell carcinoma (5–8). This ECOG study is a prospective randomized phase II trial of megestrol acetate (MA), VP-16-213, cyclophosphamide (CYC), and dianhydrogalactitol (DAG) designed to determine the antitumor activity of each of these agents against advanced renal cell cancer.

METHODS AND PROCEDURES

Metastatic disease patients with biopsy-confirmed adenocarcinoma of the kidney not previously treated with the agents used in this study were

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[2]This study was conducted by the Eastern Cooperative Oncology Group (Paul P. Carbone, Chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare. The following members participated in the study: Mayo Clinic and Mayo Foundation, Rochester, Minn (CA-13650, *R. G. Hahn*); Sidney Farber Cancer Institute, Boston, Mass (CA-23318, *M. Bauer* [*present address:* University of Pittsburgh, Pa]); Rush-Presbyterian-St. Luke's Medical Center, Chicago, Ill (CA-10948, *J. Wolter*); American Oncologic Hospital, Philadelphia, Pa (CA-18281, *R. Creech*); University of Rochester Cancer Center, NY (CA-11083, *J. M. Bennett*); and Medical College of Virginia, Richmond (CA-10572, *G. Wampler*).

Other participating institutions include: Albany Medical College, NY (CA-06594); Baystate Medical Center, Springfield, Mass (CA-20182); Boston University Medical Center, Mass (CA-11109); Brookdale Hospital Center, Brooklyn, NY; Brown University, Providence, RI (CA-15947); Case Western Reserve University, Cleveland, Ohio (CA-14548); Chicago Medical School, Ill (CA-14144); Hahnemann Medical College, Philadelphia, Pa (CA-13611); University of Florida-Jacksonville (CA-19819); Jefferson Medical College, Philadelphia, Pa (CA-14215); Johns Hopkins Oncology Center, Baltimore, Md (CA-16116); University of Manitoba, Winnipeg; Medical College of Virginia, Richmond (CA-10572); University of Minnesota, Minneapolis (CA-20365); Mt. Sinai Medical Center, New York, NY (CA-17152); National Cancer Institute, Bethesda, Md; Northwestern University Medical Center, Chicago, Ill (CA-17145); University of Ottawa, Ontario; Centre Hospitalier de Bobigny, Paris, France (CA-19273); Pennsylvania Hospital, Philadelphia (CA-13613); Hospital of the University of Pennsylvania, Philadelphia (CA-15488); University of Pittsburgh, Pa (CA-18653); Roswell Park Memorial Institute, Buffalo, NY (CA-12296); University of Pretoria, South Africa (CA-21692); Tufts University, Walpole, Mass (CA-07190); Natalie Warren Bryant Cancer Center, Tulsa, Okla; Wisconsin Clinical Cancer Center, Madison (CA-21076); and Yale School of Medicine, New Haven, Conn (CA-06510).

[3]The data coordination efforts of Barbara Buchheit, Deborah Steele, and Bonnie Zimmer are gratefully appreciated.

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considered eligible if, at the time of study entry, they had an ECOG performance status of ≥ 3, adequate hepatic and bone marrow function, and no major surgical procedures in the previous 3 weeks or chemotherapy or radiotherapy in the previous month.

Patients were stratified according to prior treatment with progestins, the presence or absence of pulmonary lesions, and performance status (ECOG 0 or 1 versus 2 or 3). Patients who had not previously received progestin therapy were randomly assigned to one of the four treatment regimens, while patients who had previously received progestin therapy were randomized to receive VP-16-213, CYC, or DAG. MA was given orally at a dose of 150 mg/m^2 daily in three divided doses. VP-16-213 was given iv at a dose of 125 mg/m^2 on Days 1, 3, and 5 every 28 days. CYC was given iv at a dose of 1000 mg/m^2 on Day 1 every 28 days. The original dose schedule for DAG was 35 mg/m^2 given iv on Days 1–5 every 35 days. After 4 months of patient entry, this dose was reduced to 25 mg/m^2 due to cumulative myelosuppression.

Platelet and wbc counts were scheduled weekly. A 25% dose reduction for VP-16-213, CYC, and DAG was provided if the wbc count nadir was between 1000 and 2000/mm^3 or if the platelet count nadir was > 75,000/mm^3. If the platelet count nadir was between 50,000 and 75,000/mm^3, VP-16-213, CYC, and DAG were given at 50% of the initial doses. If the wbc count nadir was < 1000/mm^3 or if the platelet count nadir was < 50,000/mm^3, treatment was delayed until the counts returned to normal, at which time 50% of the initial dose was given.

Patients failing to respond to MA were randomly assigned to receive VP-16-213, CYC, or DAG. Patients failing to respond to initial treatment with VP-16-213, CYC, or DAG were assigned to receive MA if they had not received progesterone therapy previously. Patients failing to respond to initial treatment with VP-16-213 or CYC who had received progesterone therapy previously were assigned to receive DAG. A third treatment step with DAG was provided for patients who failed to respond to the first and second treatments and who had not received DAG previously. Response to treatment (complete or partial), stability of disease, and progression were classified according to the accepted ECOG response criteria.

PATIENT CHARACTERISTICS

One hundred and sixty-one patients were entered in this study between January 1975 and November 1976. Seventeen patients were excluded from the analysis: five patients were withdrawn from the study before receiving protocol treatment, three were not randomized, one had no measurable disease, and followup information was not received for the remaining eight.

One hundred and six (74%) of the 144 evaluable patients had received no prior progesterone therapy, 114 (79%) had pulmonary lesions, 94 (65%) entered the study with an ECOG performance status of 0 or 1, and 86 (60%) had tumors classified as Broders' grade I or II (well-differentiated or moderately differentiated histopathology). The median age at study entry was 56 years (range, 27–79 years); the median time from onset of primary symptoms to study entry was 9 months (range, 0 months to 11 years). The 144 evaluable patients were assigned to the initial treatments as follows: MA, 37 patients; VP-16-213, 36 patients; CYC, 36 patients; and DAG, 35 patients. Twenty patients were randomized to receive a second regimen after failing to respond to MA (seven were assigned to VP-16-213, eight to CYC, and five to DAG). Forty-eight patients with no previous progesterone therapy were crossed over to MA after failing to respond to initial therapy with VP-16-213 (19 patients), CYC (18 patients), or DAG (11 patients). Four patients previously treated with progesterone were crossed over to DAG after failing to respond to initial therapy with VP-16-213 or CYC. Twelve patients received DAG as a third step after failing to respond to their first and second treatments.

TOXICITY

Five episodes of life-threatening toxicity associated with protocol therapy were reported. Three patients receiving DAG experienced life-threatening thrombocytopenia (platelet count nadirs, 14,000–22,000/mm^3 on Days 18–25). Two of these patients recovered; the third also developed leukopenia and died with septicemia. The death of a fourth patient receiving DAG was presumed to be due to pneumonitis subsequent to leukopenia (recorded wbc count nadir, 2900/mm^3). One of the patients who recovered had received DAG at the original dose of 35 mg/m^2; the other three patients received the lower dose. The fifth episode of life-threatening toxicity was septicemia experienced by a patient receiving CYC as crossover therapy subsequent to MA. Recorded wbc counts were normal and the patient recovered.

RESULTS

Three patients had objective responses to their initial therapy (one complete response to VP-16-213 lasting 225 days, one partial response to MA lasting 113 days, and one partial response to CYC lasting

125 days). Three partial responses were observed on crossover (two with MA lasting 45 and 134 days and one with CYC lasting 57 days). No responses were observed in patients treated with DAG, either as initial or crossover therapy. No responses were observed among those patients treated with progesterone prior to study entry. Five of the six primary or secondary treatment responses were observed in patients in whom metastatic pulmonary disease was the site of disease regression. The other remaining tumor response was in a patient whose soft tissue disease was the site of measurable metastatic disease and response.

Multivariate regression analyses of time to progression and death were performed in order to adjust for any imbalances within treatment groups with respect to the stratification variables and other factors such as histologic grade, prior treatment (resection of primary, radiotherapy, progesterones), sex, age, race, and time from onset of primary symptoms to study entry. After adjusting for the variables which significantly affect time to progression and survival, there were no significant differences among treatments. Patients with a good performance status (ECOG 0 or 1) and a long interval from their primary symptoms to study entry had a longer time to progression and death than those with a poorer performance status or a shorter interval from onset of primary symptoms. Patients with unresected primary tumors had a shorter time to progression and death, although given the small number of patients in this subgroup this result should only be considered as suggestive. Patients developed progressive disease rapidly with all four regimens. The overall median time to progression was 10 weeks for patients who had not previously received progesterones and 6 weeks for those who had previously received progesterones prior to study entry.

DISCUSSION

A number of the commercially available and several of the investigational antitumor agents have had limited trials in renal cell cancer. The low order of response reported herein with VP-16-213 or DAG (investigational agents) and CYC or MA (commercially available agents) is consistent with limited response rates observed in association with other reported treatment trials. Since only six objective responses were observed in 144 evaluable patients, it is apparent that none of the four treatment regimens included in this trial provided any meaningful antitumor benefit.

The limited responses observed in association with MA therapy may reflect the weak binding affinity of progestational receptors in renal cell cancer and the possible need for larger doses of progestins to produce any increased or meaningful response rate.

Further phase II trials of promising antitumor agents are clearly needed for patients with this unresponsive malignancy.

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ANNOUNCEMENTS

American Board of Internal Medicine Examinations

The next examinations in Hematology, Infectious Disease, Nephrology, Pulmonary Disease, and Rheumatology will be held on June 17, 1980. Registration for these subspecialty examinations will begin August 1, 1979, and will continue through November 1, 1979. For those physicians who began their residency training in internal medicine before June 1, 1970, and who have < 2 years of acceptable subspecialty training, this will be the last opportunity for admission to a subspecialty examination. *Applications may be obtained from:* The American Board of Internal Medicine, University City Science Center, 3624 Market St, Philadelphia, Pa 19104 (telephone: 215-243–1500).

The American Board of Internal Medicine has announced a significant change in policy regarding its training requirements. This change will affect physicians desiring admission to a subspecialty examination who began their residency training in *internal medicine* before June 1, 1970. At present, these physicians are considered to have met the training requirements for admission to a subspecialty examination after completing 1 year of acceptable training in the subspecialty. If once informed of admission to a subspecialty examination, these physicians *will continue* to be considered to have fulfilled the Board's training requirements in that subspecialty. Beginning with the subspecialty examination offered in 1981, however, *all physicians* not previously admitted to an examination will be required to meet the same training requirements which apply to physicians beginning their residency training after June 1, 1970. These requirements currently involve 2 full years of acceptable training in the subspecialty. A complete description of the Board's policies and procedures may be obtained by directing a written request to the American Board of Internal Medicine at the above address.

Medical Oncology Society Abstracts

Available upon request are the proceedings of abstracts from presentations given at the 4th Annual Meeting of the Medical Oncology Society (President, G. Mathé) held in Nice, France, in December 1978. This Society meeting includes presentations from medical oncology researchers from France and other Western European countries also with some contributions from the US and elsewhere. *Requests should be directed to:* Cancer Therapy Evaluation Program, Bldg 37, Rm 6A17, National Cancer Institute, National Institutes of Health, Bethesda, Md 20205.

International Union Against Cancer Postgraduate Courses on Clinical Cancer Chemotherapy

The International Union Against Cancer (UICC) has in recent years organized postgraduate courses on clinical cancer chemotherapy which were held in Tripoli, Libya (1976), Istanbul and Ankara, Turkey (1977), and Montevideo, Uraguay (1977).

The courses are based on a didactic manual developed by a UICC Committee including Dr. S. Monfardini, *Chairman* (Italy), Dr. K. Brunner (Switzerland), Dr. D. Crowther (United Kingdom), and Dr. D. Olive (France) who were also responsible for designing the course structure and producing guidelines for the didactic approach to be employed. This manual, which was first produced in English, has subsequently been translated into Turkish and Spanish, and Portuguese and German versions will soon be available.

Each course lasts for 5 or 6 days and is run by an International Faculty of five or six specialists appointed by the UICC from a group of > 100 medical oncologists from 21 different countries who have offered their services in this field, and by a National Faculty of similar size nominated by the host country. The following courses will be held (date, Chairman of National Faculty and Chairman of International Faculty, respectively): Nürnberg, Federal Republic of Germany (October 1979, Dr. Gallmeyer and Dr. Märtz);

Teheran, Iran (November 17–22, 1979, Dr. Mojtabai and Dr. Olive); and Bombay, India (November 26–December 3, 1979, Dr. Jussawalla and Dr. Woolley).

For further information, please contact: David W. Reed, Assistant to the Director, International Union Against Cancer, 3 rue du Conseil-Général, CH-1205 Geneva, Switzerland.

Fifth Congress of the Medical Oncology Society

Nice, France, December 1–3, 1979

The Société de Médecine Interne Cancérologique announces the Fifth Congress of the Medical Oncology Society to be held at the Hotel Negresco, Nice, France on December 1–3, 1979. The congress will focus on the diagnosis and treatment of cancer and experimental studies. *Direct inquiries to:* Prof. M. Schneider, Centre Antoine-Lacassagne, 36 Voie Romaine, 06054 Nice, Cedex, France (telephone: [93]81.71.33 and telex: CAL NICE 470.344 F).

Symposium on Head and Neck Cancers

Baltimore, Maryland, June 15 and 16, 1979

The Program of Oncology, Department of Surgery, University of Maryland School of Medicine, Baltimore, Maryland, is sponsoring a Symposium on Head and Neck Cancers to be held June 15 and 16, 1979. *For more information contact:* Program of Continuing Education, University of Maryland School of Medicine, 10 S Pine St, Baltimore, Md 21201 (telephone: 301-528–3956).

Consensus Development Conference on "The Treatment of Primary Breast Cancer: Management of Local Disease"

Bethesda, Maryland, June 5, 1979

The National Cancer Institute (NCI) of the National Institutes of Health (NIH) is sponsoring a Consensus Development Conference on "The Treatment of Primary Breast Cancer: Management of Local Disease" to be held on June 5, 1979, from 8:00 AM to 8:00 PM at the Jack Masur Auditorium, Bldg 10, Clinical Center, NIH, Bethesda, Md. Seating is limited and preregistration is required. *For further information contact:* Ms. Betty Doolittle, Division of Cancer Treatment, NCI, Landow Bldg, Rm 8C08, NIH, Bethesda, Md 20205 (telephone 301-496–2522).

XX Advanced Course on Clinical Oncology: "Diagnostic Progress in Oncology

Milan, Italy, June 18–20, 1979

The XX Advanced Course on Clinical Oncology, entitled "Diagnostic Progress in Oncology," which is being organized by the National Institute for the Study and Treatment of Tumours and the Milan Centre of the Anti-Tumour League, will be held on June 18–20, 1979, at the Cariplo Auditorium, Via Romagnosi n. 6, Milan, Italy.

The Chairmen are Professors Umberto Veronesi and Luigi Gallone, the Coordinators are Professors Adalgiso Guzzon and Pasquale Spinelli, and the Secretary is Dr. Mario De Lena. The speakers will be French, English, Israeli, Belgian, American, Swiss, and Italian experts in specific fields. Simultaneous translations will be provided in Italian and English.

The enrollment fee is 100,000 Italian lire. Because of limited space in the auditorium, this fee should be sent as soon as possible to: American Express, Co. S.A.I., Servizio Congressi Italia, Piazza Mignanelli, 4, 00187 Rome, Italy (telephone 06/6764). For more *scientific information contact:* Lucia Manfredi, Ufficio Attività Didattiche, Istituto Nazionale Tumori, Via G. Venezian, 1, 20133, Milan, Italy (telephone: 02/2363764).

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